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PROCEEDINGS OF THE 1970 MANNED SPACECRAFT CENTER ENDOCRINE PROGRAM CONFERENCE (OCTOBER 5 to 7, 1970)

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PROCEEDINGS OF THE 1970

MANNED SPACECRAFT CENTER

ENDOCRINE PROGRAM CONFERENCE

INTRODUCTION

By Carolyn S. Leach, Ph. D.*

Man has successfully faced and overcome the challenges of the space-flight environment on short-duration missions. However, a decade after the inception of the manned U.S. space program, the medical directorate is still assessing the physiological changes that result from working in the environment of space.

A comprehensive review of the endocrinological data available to date was the subject of the NASA Manned Spacecraft Center symposium held October 5 to 7, 1970. The purpose of the symposium was to assemble various experts in their fields to assess the endocrinological changes observed in the Apollo crewmen. The attendees all have a specialized expertise that is applicable to the overall endocrine program developed for support of the Apollo missions and for the extended-duration Skylab flights.

The aims of this endocrine program are three.

1. The establishment of an operational laboratory for immediate endocrinological assays at the Manned Spacecraft Center

2. The assemblage of a group of endocrinological experts qualified to advise on procedures and the interpretation of data

3. The advancement of the field of endocrinology by the application of analytical procedures low in sample volume requirements but high in specificity

This symposium marked the first meeting of the group chosen to help conduct the NASA Manned Spacecraft Center endocrine program. Each attendee made a presentation on his specialty, then edited the transcript of his presentation, which was tape recorded for purposes of compiling this symposium report. The 10 presentations relate the authors' contributions in support of the program either with respect to studies or to methodological development.

The reader is advised that the information presented is that current at the time of the symposium and that no attempt has been made to update these presentations by inclusion of more recent data.

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1. RED-CELL-MASS AND PLASMA-VOLUME CHANGES OBSERVED IN ASTRONAUTS ON THREE GEMINI AND THREE APOLLO MISSIONS

By Philip C. Johnson, M.D.*

INTRODUCTION

Nuclear-medicine procedures were performed for three Gemini and three Apollo missions. Because these studies are the only nuclear-medicine procedures ever performed for crews who have been in a weightless environment, it is not possible to compare these results with any others. The nuclear-medicine studies were assigned to these missions as one part of the medical operational support required to guarantee the safety of the crews. Because these were operational studies rather than research studies, the number of data points was limited to the minimum number that was absolutely necessary to guarantee crew safety. The missions studies have included both the high-oxygen/zero-nitrogen atmosphere of the Gemini Program and the somewhat-lower-oxygen/partial-nitrogen atmosphere of the non-lunar-landing Apollo missions. Specimens have been obtained at the Manned Spacecraft Center, at the Kennedy Space Center, and aboard the recovery carriers. The Apollo data included ground-based controls.

DISCUSSION

Plasma-Volume Changes

Before the Mercury missions, it was anticipated that cardiovascular deconditioning would occur during the time the crews were in a weightless environment. Bedrest and water immersion are the only ways to simulate weightlessness without flying a mission. In preparation for the more prolonged Gemini missions, plasma-volume changes were studied

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during bedrest and water immersion of various durations designed to simulate the effects of weightlessness. The results of one of the studies are contained in table 1-I, in which it is shown that mean plasma volume decreased 563 milliliters during 12 hours of water immersion, 318 milliliters during bedrest, and only 151 milliliters during chair rest for similar periods. This loss of plasma volume resulted in a loss of circulatory albumin and an increased output of urine.

The Manned Spacecraft Center results were combined with published studies of plasma-volume changes produced by bedrest (fig. 1-1). Although the studies shown were not designed to be completely alike, the differences in design among the investigators were relatively minor. The figure indicates that a mean 10-percent decrease in plasma volume was experienced by the second day of bedrest. By the 27th day, plasma volume decreased to 20 percent of the control value. Plasma volume returned to normal after a period of bedrest, but the recovery took more time than the decrease. It must be remembered that for each of these studies considerable blood was drawn from the individuals, a factor that may have influenced the results obtained.

These results are compared in table 1-II with those obtained after the non-lunar-landing missions. The mean percent change after each mission is compared with the percent change expected if the changes in the missions were to be similar to the bedrest data shown in figure 1-1.

Certainly these data do not suggest that plasma-volume changes greater than those predicted from bedrest would be expected in continued space flights up to at least 14 days. The increase in plasma volume after the Gemini VII mission may represent a compensatory response to the red-cell-mass decrease found during that mission or it may be that the effect on plasma volume is self-limited.

Red-Cell-Mass Changes

The red-cell-mass changes during the Gemini and Apollo missions will be discussed next. The red-cell mass is a more stable volume than is the plasma volume. Red cells cannot be mobilized as can plasma proteins; therefore, losses are replaced slowly. Factors changing both red-cell production and death rate affect the total circulating red-cell mass; these factors include bedrest, altitude, physical conditioning, disease, and drugs.

Bedrest probably causes a decrease in red-cell mass, but such changes during bedrest are not as well documented as plasma-volume changes because only in recent bedrest studies have red-cell-mass determinations been performed. Oberfield et al. (ref. 1-1) noted an 8.6-percent mean

increase in the red-cell mass of orthopedic patients who became ambulatory after prolonged immobilization. The mechanism for these changes is unknown.

Changes in red-cell mass following altitude changes are well documented; however, this statement needs to be qualified in that what is well known is the changes resulting from decreases in oxygen tension. Not as well documented are the potential changes resulting from a hyperoxic low-pressure environment. The atmosphere used during the Gemini and Apollo missions was 100 percent oxygen at 258 torr. This atmosphere produces a total pressure equivalent to 8500 meters, but the oxygen pressure is 60-percent greater than that found at sea level. Generally, studies before the start of the manned flights did not suggest that this atmosphere would cause changes in the red-cell mass — with one exception. Helvey (ref. 1-2), using hematocrit changes to estimate red-cell-volume changes, showed marked drops in hematocrit when 100 percent oxygen at 363 torr was used. He described this change, which occurred in 48 hours, as a hemolytic process.

Red-cell-mass changes noted during six missions are shown in table 1-III. All six astronauts in the Gemini missions had a decrease in red-cell mass. If the quantity of red cells lost (an average of 319 milliliters) is converted to quantity of blood, each of the six astronauts lost approximately 700 milliliters of blood.

Similar studies were performed after the Apollo 7, 8, and 9 missions. The 7-percent mean decrease in the three Apollo 9 crewmembers is a greater change than the percent change in five of the six Apollo 7 and 8 crewmembers. However, the Apollo 9 crew percent changes are less than those of the six Gemini crewmembers. The oxygen pressure in the atmosphere was significantly greater in the Gemini missions than in the Apollo 7 and 8 missions. (The Apollo 9 oxygen pressure was of an intermediate value.) Nitrogen remained in the Apollo 7 and 8 command module atmospheres. The nuclear-medicine study group believes that the presence of nitrogen may have moderated the red-cell loss observed in the Gemini missions. As may be recalled, nitrogen was not included in the spacecraft atmosphere until after the January 1967 command module fire. In all missions since, a nitrogen-containing atmosphere has been used during the launch phase. After lift-off, gas losses are replaced by oxygen so that the atmosphere gradually approaches 100 percent oxygen. Because the dump rate is low, the atmosphere never reaches 100 percent unless the hatches are opened (as was done halfway through the Apollo 9 mission, but not on the Apollo 7 and 8 missions). If the red-cell-mass decreases on the missions in which the atmosphere did not include nitrogen are combined and compared with the two missions in which nitrogen was present, a mean decrease in red-cell mass of 13 percent results, compared with a 2.0-percent decrease for those when nitrogen was present. These means are different at the <0.01 level.

Because no bleeding was experienced, two mechanisms exist that could explain the decrease in red-cell mass observed in the Gemini and Apollo 9 studies — increased intravascular destruction and decreased production rate.

Certain other studies were performed to attempt an etiological classification of the change. These included chromium-51 red-cell-survival studies, the results of which are shown in table 1-IV. The mean-red-cell half-times of the two Gemini missions and of the Apollo 9 mission are shorter than the expected or observed norms. The data are sketchy, but certainly no evidence exists of a difference between six crewmembers and six controls for the Apollo 7 and 8 missions, in contrast to the Apollo 9 mission on which the lowest mean is observed. This difference suggests that a mild hemolysis occurred during the high-oxygen missions. However, the differences are slight.

Several explanations for the mild hemolysis observed on the Gemini missions have been offered, the most plausible of which suggests that the hyperoxic environment produces a relative tocopherol deficiency. Increased oxygen tension is known to cause red-cell abnormalities. However, these abnormalities have only been reported at oxygen tensions higher than 100 percent oxygen at 258 torr. High-oxygen tensions are thought to cause hemolysis through peroxidation of membrane lipids. This effect is counteracted by vitamin E. Some evidence exists that the astronauts' prepackaged diet was slightly deficient in tocopherol during the Gemini missions.

The plasma levels of vitamin E and vitamin A of the Apollo 9 crew and a control group are shown in figure 1-2. The ground-based controls showed no change or an actual increase in vitamin A during the mission, while the crew showed decreases in both vitamins E and A, an observation that tends to support the postulate of a tocopherol deficiency.

With decreased red-cell mass and decreased red-cell survival, increased iron turnover would be expected if the bone marrow were responding to this stress. The iron-turnover data obtained from the Apollo 7 and 8 missions are listed in table 1-V.

The crew had higher serum irons, slower half-times, lower iron turnovers, and lower percent reappearance. Although each of these values is interdependent, decreased bone-marrow activity at the time of recovery is suggested. These iron-study values suggest inhibition of bone-marrow function rather than hemolysis. However, the Apollo 7 and 8 flights were low-oxygen missions and the data may not be indicative of the changes in the Gemini missions and the Apollo 9 mission.

The degree to which these volume changes affect the endocrine system are of interest. Both a decreased plasma volume and a decreased red-cell

mass at the time of recovery were observed. The red-cell-mass change has been associated with decreased red-cell survival and relatively low iron kinetics at the time of recovery in low-oxygen missions.

Fluid-Volume Changes

In addition to decreased blood volume, the crews exhibited decreased extracellular fluid volume and perhaps changed intracellular fluid volume. At the time of return, the crews weighed approximately 3 kilograms less than they did at lift-off; about 67 percent of this weight loss was recovered in the first 24 hours after their return. The weight that was not regained is presumed to represent the effects of the suboptimal caloric intake chosen by the crew. During that time, they lose their intolerance to lower-body negative pressure and recover most of their exercise tolerance. The profound weight loss has not been seen in the bedrest studies. However, mean extracellular losses of 1.1 liters at 4 days and 2.2 liters at the end of 10 days of bedrest have been measured. Although these measurements have not yet been made for the flight crews, the crew weight losses suggest that the extracellular fluid decreases more rapidly during a mission than during bedrest.

Orthostatic intolerance would be expected when the crew returns to a one-g environment, with decreased plasma volume as a contributory factor. Extracellular fluid volume should be decreased, but it has not yet been measured. These volume changes should be associated with increased renin production, with increased epinephrine output, and eventually with increased corticoid production. Antidiuretic hormone activity would increase. The results of these changes would be water and sodium retention accompanied by a potassium diuresis. The white count should increase as should the blood sugar. Indeed, water retention and sodium retention have been observed with increased white-blood-cell counts and blood sugars, but potassium diuresis is absent.

CONCLUDING REMARKS

How the longer duration of the Skylab missions will affect these parameters as the crews return to a one-g environment cannot be answered at this time. An additional unknown is the effects of the larger living volume and the exercise planned for the Skylab missions. No longer will the crew be confined to their couches as in the Mercury and Gemini missions. Although the Apollo command module permits movement in the cabin, the astronauts have never been assigned the exercise profile that should be one part of the Skylab Program. This exercise should allow them to

maintain muscle strength, even on these long-duration missions. How this exercise will affect the orthostatic intolerance is unknown.

It is interesting to note that the longest mission to date, Gemini VII, was not associated with a decreased plasma volume; yet other changes characteristic of orthostatic intolerance were observed. The Skylab atmosphere will not be hyperoxic, and, unlike the Gemini atmosphere, will contain nitrogen. The exercise program should be an additional stimulus to maintain the crew's red-cell mass. It is expected that these changes should moderate or even prevent the red-cell-mass changes. In addition, the Skylab food will probably contain more vitamin E. However, as was the case with the plasma-volume changes, whether this prediction will be true will not be known until the Skylab data are analyzed.

With respect to the nuclear-medicine procedures scheduled for the Skylab missions, permission to perform the studies just described has been sought. In addition, hopefully, the total body water, total exchangeable potassium, and extracellular space can be determined. These studies will be designed to allow more precise interpretation of the expected endocrine changes.

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TABLE 1-I.- CHANGES NOTED IN

WEIGHTLESSNESS SIMULATIONS

Mean change	Water immersion	Bed-rest	Chair rest
Plasma volume, ml	-563	-318	-151
Vascular albumin, ml . . .	-53	-32	-2
Urine, ml	935	674	452
Hematocrit, percent	8	4	3

TABLE 1-II.- PLASMA-VOLUME PERCENT CHANGE,

APOLLO AND GEMINI MISSIONS

Mission	Duration, days	Change, percent	
		Mean	Predicted
Gemini IV	4	-8	-7
Apollo 8	7	-13	-9
Gemini V	8	-7	-10
Apollo 7	11	-3	-11
Apollo 9	11	-8	-11
Gemini VII	14	11	-12

TABLE 1-III.- RED-CELL-MASS PERCENT CHANGE,

GEMINI AND APOLLO MISSIONS

Mission	Duration, days	Change, percent			
		Astronaut			Mean
		1	2	3	
Gemini IV	4	-12	-13	--	-12
Gemini V	8	-20	-22	--	-21
Gemini VII	14	-19	-8	--	-14
Apollo 9	11	-4	-7	-10	-7
Apollo 7	11	-1	-0	-9	-3
Apollo 8	7	2	-2	-4	-1

TABLE 1-IV.- MEAN RED-CELL SURVIVAL, GEMINI AND APOLLO MISSIONS

[Half-times using chromium-51]

Mission	Mean half-time, days	
	Crew	Control
Gemini V	18	--
Gemini VII	23	—
Apollo 9	24	28
Apollo 7	26	16
Apollo 8	31	30

TABLE 1-V.- IRON KINETICS AT RECOVERY

Parameters	Crew	Controls
Serum iron, μg	105 ± 6	94 ± 4
Half-time, min	138 ± 7	112 ± 5
Iron turnover, mg/kg per body weight/day	.34 ± .03	.41 ± .03
Reappearance, percent	86 ± 2	95 ± 3

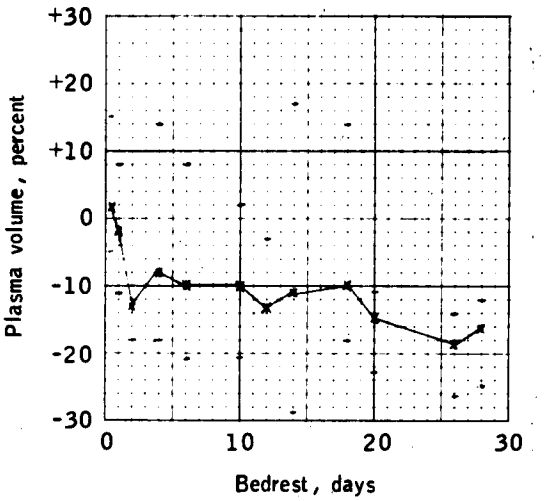
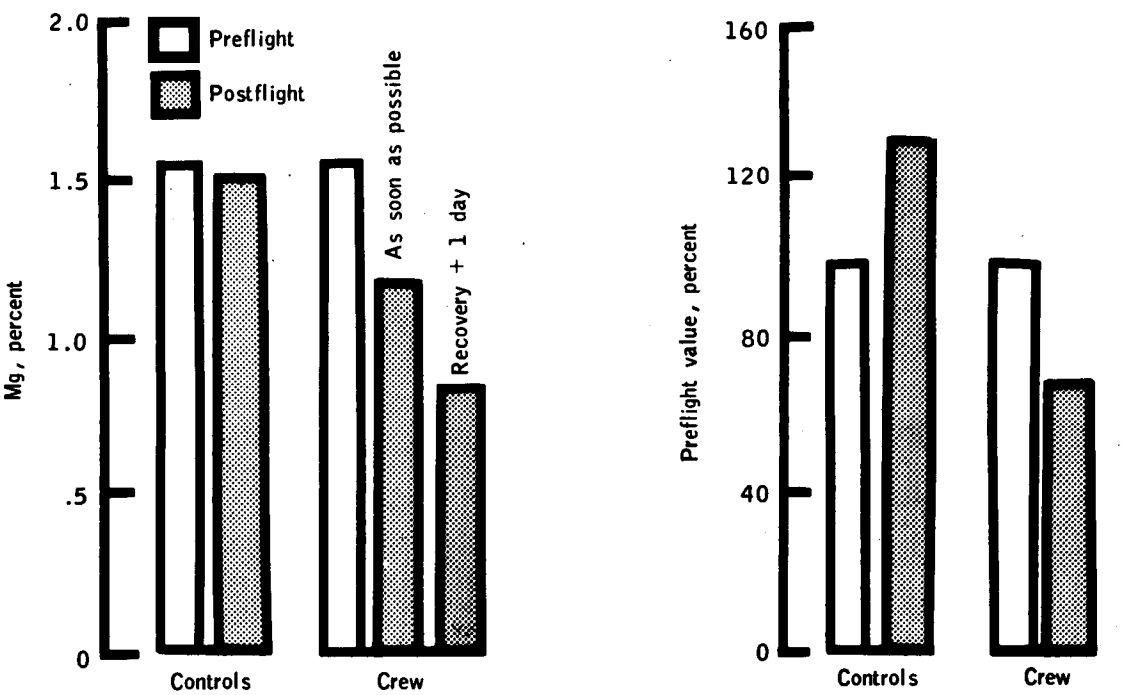


Figure 1-1.- Percent decrease in mean plasma volume during bedrest.



(a) Plasma vitamin E.

(b) Plasma vitamin A.

Figure 1-2.- Plasma levels of vitamins E and A in control group and in Apollo 9 crew.

2. HEMATOLOGY RESULTS ON GEMINI AND APOLLO MISSIONS

By Craig L. Fischer, M.D.* and Stephen L. Kimzey, Ph. D.†

HEMATOLOGICAL PROGRAM

The best summaries of what was known about red-cell mass and plasma volume, after the Gemini Program was terminated, are contained in figures 2-1 and 2-2, respectively. The plasma-volume changes (fig. 2-2) seem to stabilize by the fourth day of space flight and do not progress between the fourth and the eighth flight days. A significant decrease in the red-cell mass (fig. 2-1) does not seem to occur after the eighth day, but a compensatory increase (on at least one Gemini mission) in plasma volume occurred, such that the preflight blood volume was regained by the 14th flight day. In addition to these changes, the observation was made during the Gemini Program that the red-cell mass was significantly decreased by space flight and that this red-cell-mass decrease was associated with an increase in the mean corpuscular volume, an increase in the osmotic fragility of the red-cell population, and an increase in the postflight reticulocyte counts.

The medical portion of the Apollo Program was designed to investigate in more detail the changes observed during the Gemini Program. Some of the medical data obtained during the Apollo missions to date are shown in table 2-I. The same types of hematological changes were expected in the Apollo Program as had been observed in the Gemini flight series. Surprisingly, no decrease in the red-cell mass was observed during the Apollo 7 or 8 missions.

A change in launch-atmosphere composition — from 100 percent oxygen to 60 percent oxygen and 40 percent nitrogen at 260 torr with cabin leakage being replaced by oxygen — was effected after the tragic Apollo fire. Therefore, the Apollo 7 and Apollo 8 missions were characterized by an oxygen concentration that never closely approximated 100 percent;

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in fact, it reached only 90 to 94 percent. Although some inconstant changes in phosphofructokinase, hexokinase, and phosphoglyceric kinase were observed, these changes were not really interpretable. Otherwise, no significant changes were observed during the Apollo 7 and 8 missions, other than the slight, but statistically significant, reduction in the epythpopoietic activity, as measured by ferrokinetic studies.

On the Apollo 9 mission, the crew opened the spacecraft hatches to perform extravehicular activity. After the spacecraft had been resealed, and pressurized with 100 percent oxygen, the crew denitrogenated and lived in a pure-oxygen environment for the remaining 4 to 5 days. Even though crew denitrogenation began at the time of repressurization and the crew lived in 100 percent oxygen for the next 5 days, only a 7-percent mean decrease in crew red-cell mass was observed — a significant, but not dramatic, change. However, the crew was not denitrogenated before the mission as had been the crews in the Gemini Program. Gemini crewmen breathed 100 percent oxygen at 760 torr for 3 hours before the mission, again on the launch complex for several hours before liftoff, and then proceeded with a mission on which a 100-percent-oxygen, 260-torr atmosphere was used. Thus, the Apollo 7 and 8 oxygen-nitrogen profiles differed completely from the Gemini missions. Even the atmosphere profile of the Apollo 9 mission differed considerably, although it was somewhat similar to the Gemini-type atmosphere profile.

The Apollo 9 mission is discussed at length in this section, because it was the only Apollo mission on which 100 percent oxygen at 260 torr was used for a significant length of time and for which detailed studies are available. A decrease in the reticulocyte count was observed, but no opportunity existed to perform the ferrokinetic studies. Other observations included a significant loss in red-cell mass, a significant decrease in plasma tocopherol vitamin E, and a significant decrease in plasma vitamin A. Some changes in the enzymes were measured by Mengel (ref. 2-1) at Ohio State University. (On the Apollo 10, 11, 12, and 13 missions, the 100-percent-oxygen atmosphere was not used, and no decreases in tocopherol and vitamin E were observed.)

As shown in figure 2-3, significant decreases were observed after the Apollo 9 mission in the plasma vitamin E and vitamin A levels, as compared with three controls. The reductions in the plasma vitamin E are statistically significant. Concomitant changes in the red-cell-membrane vitamin E or vitamin A were not observed.

Total phospholipid, neutral lipid, and fatty acids of several major phospholipids of the red-cell membrane were measured. The red-cell lecithin totals (as measured by thin-layer separation) of the Apollo 9 crew and the controls are shown in figure 2-4. No significant changes were noted in the controls; however, a very marked change in the red-cell-membrane lecithin levels of the oxygen-exposed crew was observed.

This very significant change was not related to diet. The fatty-acid patterns of this phospholipid and others show a shortening of the fatty-acid chains, particularly the long-chain, unsaturated fatty acids, such as C_{24}, C_{22}, and C_{18}. In other words, what was observed is compatible with lipid peroxidation. Thus, not only was there a quantitative change in the phospholipids but also a qualitative change in the fatty acids of the phospholipids. Evidently both changes can be explained on the basis of peroxidation.

On the same mission, the sodium-potassium flux on the red cell was studied; the results are shown in figure 2-5. Before the mission, immediately afterward, and 1 day after recovery, the controls showed essentially no change, but a significant reduction in the active component of potassium flux was observed in the oxygen-exposed flight personnel. Only active cation transport was measured. Only because logistics are a severe problem, the passive limb of cation transport was not studied.

In summary, the Apollo 9 data indicated reductions in plasma tocopherol, plasma vitamin A, and several major red-cell phospholipids. Lecithin, which is a major component of the red-cell membrane, not only showed a dramatic change quantitatively, but qualitatively as well. A reduction in the active cation transport of red cells was also observed after the Apollo 9 mission.

With respect to the overall Apollo hematological program (table 2-II), no significant red-cell-mass changes were observed after the Apollo 7 and 8 missions; however, a modest but significant loss of red cells was observed after the Apollo 9 mission, presumably as a result of the different spacecraft atmosphere. The significant reduction of red-cell mass after the Apollo 9 mission was associated with decreases in plasma tocopherol and vitamin A. Decreases in red-cell-membrane phospholipid content and in the active sodium-potassium flux of the cells were also observed. Morphologic changes (acanthocytes) were shown by wet-cell preparations and phase microscopy. Such changes are well known; Ways and Hanahan (ref. 2-2) have reported that, in acanthocytes, alteration of the sphingomyclin-to-lecithin ratio occurs, as well as qualitative changes in the phospholipids of the red-cell membrane. Again, the difference between the Apollo 9 data and the Apollo 7 and 8 data is oxygen or atmosphere related.

All the data are integrated into a hypothesis that is diagramed in figure 2-6, which includes the Jacob's model (ref. 2-3) of hereditary spherocytosis, some of the work by Mengel (ref. 2-1), and some of the concepts that have evolved at the Manned Spacecraft Center (MSC) laboratory. Figure 2-6 is not necessarily original, but it is a synthesis of already proven and accepted concepts presented in the manner that seems to fit the MSC data best. It is believed that hyperoxia causes peroxidation of the red-cell-membrane lipids, as Mengel (ref. 2-1) has

shown. This peroxidation of red-cell-membrane lipids can cause one of several results. (1) The plasma vitamin E and vitamin A levels can be reduced just by virtue of the fact that these sterols are lipid antioxidants and are consumed in this type of reaction. (2) Peroxidated lipids can physically compromise red-cell-membrane integrity. Lipid peroxides are very effective and efficient red-cell-membrane sulfhydryl-group inhibiters. Thus, if red-cell lipid peroxides were formed, inhibition of red-cell-membrane sulfhydryl groups could be expected. The sulfhydryl groups are quite important in maintaining the integrity of passive red-cell-membrane cation transport. If active cation transport is poisoned by the same mechanism, one would observe osmotic swelling of the red cells resulting in attainment of critical volume and lysis. Thus, altered active and passive transmembrane cation transport may be occurring simultaneously. Because the data fit the hypothesis so well, it is believed that the hemolytic event has been described accurately and that it is oxygen related.

Personnel at the MSC Clinical Laboratories have functioned as a Federal resource group and have had the opportunity to apply a very comprehensive hematological evaluation in several areas, such as space flight, undersea exploration, and vacuum-chamber tests, to name just a few. The same protocol was performed by the same investigators using the same techniques in different environments. The supported programs included Tektite I and II, the Apollo 7, 8, and 9 missions, an MSC vacuum-chamber test called 2TV-1, two Brooks Air Force Base chamber tests, a Sealab III chamber test, and the Gemini IV, V, and VII missions. In table 2-III, these tests are ranked according to the mean red-cell-mass loss that was measured in the subjects. These data include the percent loss, the atmosphere composition, the number of subjects, and the exposure duration. What is noteworthy is that, anytime a 100-percent-oxygen atmosphere was used, significant red-cell-mass loss occurred. Anytime any diluent gas was present (particularly nitrogen), no significant red-cell loss was observed. This effect suggests a protective effect of nitrogen, more specifically an active mechanism.

Several nitrogen effects can be cited from the literature. Nitrogen is known to participate actively in the formation of clathrates and liquid crystals and, thus, can stabilize red-cell-membrane water. The phenomenon can be thought of in terms of molecules of water becoming more organized and more ordered and, thus, more restricted in their degrees of freedom. Such an occurrence would be directly related to membrane transport, which is a physical-chemical phenomenon. The extent to which nitrogen provides a protective mechanism in red-cell-mass loss cannot be defined yet, but it is evident that nitrogen is protective.

It also appears that tocopherol is protective. Several methods to test this hypothesis exist, when the basic mechanisms are understood. Tocopherol will not be a limiting factor, even if a 100-percent-oxygen

atmosphere must be used for long periods of time. However, appropriate immediate action could be taken to avoid any problems that might arise. Thus, one of the more interesting findings, if it proves true, of the MSC hematology program is the very strong, protective, active effect of nitrogen, which has always been called a nerve gas.

BIOCHEMICAL PROGRAM

Some of the biochemical data for the Apollo 7 to 13 missions are shown in table 2-I. Each arrow represents a standard deviation. The overall postflight data for the missions are plotted in the right-hand column. It must be remembered that the mission profiles differ and, therefore, it may not be accurate to plot the overall trend in this manner. The only consistent trend is a significant postflight increase in true serum glucose, which is believed to be an epinephrine-related event. This increase is transient and usually disappears by the time of the next blood sampling (usually 12 to 24 hours later), when the glucose is usually normal. An apparent consistent decrease is observed in the serum cholesterol and uric acid, which may be related to diet in that the crews are eating a better diet during the missions than they do normally. This decrease is very slight, but significant at the level of one standard deviation. A consistent change in serum creatinine was observed on only two missions. Whether this decrease indicates some alteration of renal clearance or not is not known, because, unfortunately, renal-clearance tests are not currently performed aboard the recovery ship.

With respect to isoenzymes, although the changes are somewhat inconsistent, a slight overall decrease in lactic dehydrogenase (LDH) 4 and a slight increase in LDH 5 were observed. These changes may be related to logistics of sample stabilization and are only apparent. No other enzyme exhibited this change. It is interesting to note that hemolysis was experienced on the Apollo 9 mission. The LDH R is a red-cell-related isoenzyme; this isoenzyme was elevated by three or more standard deviations on the Apollo 9 mission. The LDH R is not quite as elevated, if at all, on the other missions. Sodium, potassium, chloride, and osmolality do not seem to show any consistent changes. Sometimes, significant increases occurred; sometimes, significant decreases. The total protein and electrophoretic patterns indicate some trends, but the trends are very difficult to interpret. Changes in glucose, cholesterol, uric acid, LDH 4, and LDH 5 were observed; these changes may be logistically related. Two epinephrine-related factors — absolute neutrophilia and red-cell changes — have been reported.

In summary, the biomedical data are not significantly helpful, no matter how detailed the study. The least that can be said objectively

with certainty is that no significant changes in the serum biochemistry occurred. Alkaline-phosphotase isoenzymes and iodized calciums will be measured for the first time on the Apollo 14 mission. These data will be of interest in relation to the bone-mobilization cluster.

The Apollo Program biochemical data indicate the following.

1. A postflight hyperglycemia is regularly observed as the result of an increased release of catecholamines that are secondary to the stress of entry.

2. Serum-cholesterol and uric-acid levels generally decline during the flights, presumably as the result of the flight diet.

3. A transient postflight decrease in LDH 3 (associated with an increase in LDH 4) is often observed.

4. A transient postflight decrease in total serum carbon dioxide has been observed in the one crew (Apollo 13) tested for this parameter.

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TABLE 2-I.- SUMMARY OF BIOCHEMICAL DATA — APOLLO PROGRAM

Parameter	Mission							
	Apollo 7	Apollo 8	Apollo 9	Apollo 10	Apollo 11	Apollo 12	Apollo 13	AOA (a)
Glucose	[b]↑↑↑	[c]↑↑	[d]↑	↑↑↑				↑↑
Cholesterol	[e]↓	[f]↓↓	↑	↓			↓	↓
Serum glutamic-oxalacetic transaminase								
Blood urea nitrogen				↑↑			↑	
Uric acid	↑	↑↑	↑	↑↑	↑↑		↑	↑
Alkaline phosphatase			↑↑↑					
Calcium								
Magnesium				↑↑	↑↑			
Inorganic phosphate		↑↑	↑↑↑	↑↑	↑↑			
Total bilirubin		↑		↑↑	↑↑	↑	↑↑	
Creatinine	↑↑							↑↑
Creatinine phosphokinase		↑↑	↑↑			↑	↑	
Lactic dehydrogenase (LDH)	↑↑		↑↑	↑↑	↑↑			
English nomenclature								
LDH 1 } Heart fraction								
LDH 2 } Heart fraction		↑↑	↑↑↑	↑↑	↑↑		↑	
LDH 3			↑↑		↑↑			
LDH 4		↑↑	↑↑		↑↑↑			
LDH 5 — Liver fraction				↑↑				
Sodium	↑↑	↑	↑↑	↑↑	↑↑		↑	
Potassium	↑↑	↑						
Chlorine	↑	↑		↑↑		↑		
Osmolality	↑↑	↑↑	↑↑	↑↑	↑↑			
Total protein			↑↑	↑	↑↑		↑	
Albumin			↑↑	↑				
Alpha 1			↑↑	↑↑				
Alpha 2	↑		↑↑	↑↑	↑↑			
Beta				↑↑				
Gamma				↑↑				

[a] Apollo over all; cumulative data from all Apollo missions to date.

[b] ↑↑↑ 3σ positive deviation.

[c] ↑↑ 2σ positive deviation.

[d] ↑ 1σ positive deviation.

[e] ↓ 1σ negative deviation.

[f] ↓↓ 2σ negative deviation.

TABLE 2-II.- RADIOISOTOPE-HEMATOLOGY — APOLLO PROGRAM

Parameter	Mission			
	Apollo 7	Apollo 8	Apollo 9	Apollo 10
	Parameter trend			
Plasma volume	0	[a]↓↓	[b]↓	--
Red-cell mass	0	0	↓	--
Ferrokinetics	↓	↓	--	--
^{14}Carbon-glycine survival	0	0	0	--
^{51}Chromium survival	0	0	↓	--
Active red-cell sodium-potassium flux	--	--	↓	0
Passive red-cell sodium-potassium flux	--	--	[c]0	[c]0

[a]↓↓2σ negative deviation.

[b]↓1σ negative deviation.

[c]Technically unsatisfactory data.

TABLE 2-III.- ^{51}CHROMIUM RED-CELL-MASS DATA

Study or mission	Atmospheric profile		Number of subjects	Exposure period, days	Red-cell-mass change, percent	
	Pressure range, torr	Gas			Range	Mean
Tektite I	160 876	Oxygen Nitrogen	4	60	+8.0 to -7.4	-2.0
Apollo 8	237 to 304 21 to 456	Oxygen Nitrogen	3	7	+2.0 to -4.0	-2.0
Apollo 7	237 to 304 21 to 456	Oxygen Nitrogen	3	11	-2.0 to -9.0	-3.0
2TV-1	237 to 304 21 to 456	Oxygen Nitrogen	3	11	-1.6 to -7.3	-3.0
Brooks Air Force Base chamber study	236 23	Oxygen Nitrogen	4	21	+6.0 to -10.0	-3.0
Sealab III chamber study	209 836 12 887	Oxygen Nitrogen Helium	3	12	+4.0 to -9.0	-5.0
Apollo 9	258 to 304 0 to 456	Oxygen Nitrogen	3	10	-4.0 to -10.0	-7.0
Brooks Air Force Base chamber study (1970)	260	Oxygen	8	30	-7 to -22	-12.7
Gemini IV	288	Oxygen	2	4	-12.0 to -13.0	-13.0
Gemini VII	258	Oxygen	2	14	-8.0 to -19.0	-14.0
Gemini V	258	Oxygen	2	8	-20.0 to -22.0	-21.0
Philadelphia chamber study (Tektite II)	158 2 882	Oxygen Nitrogen	6	14	-19 to -31	-27.0

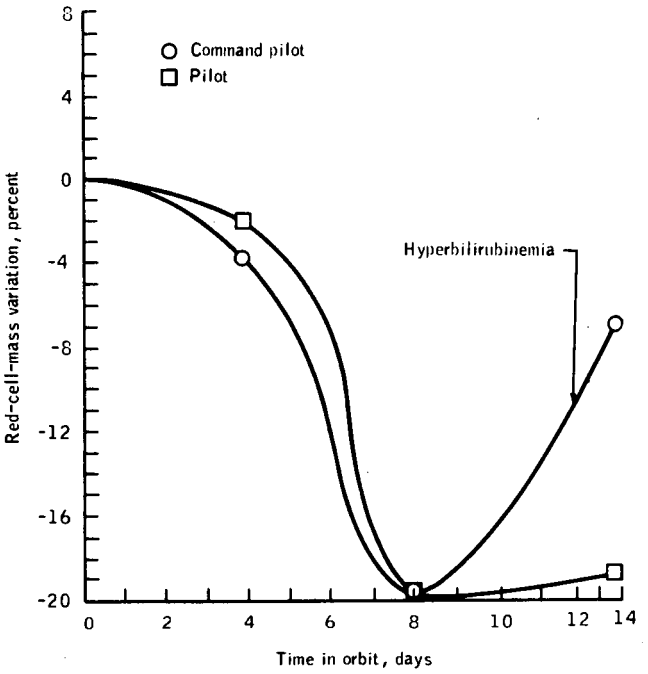


Figure 2-1.- Gemini Program red-cell-mass data.

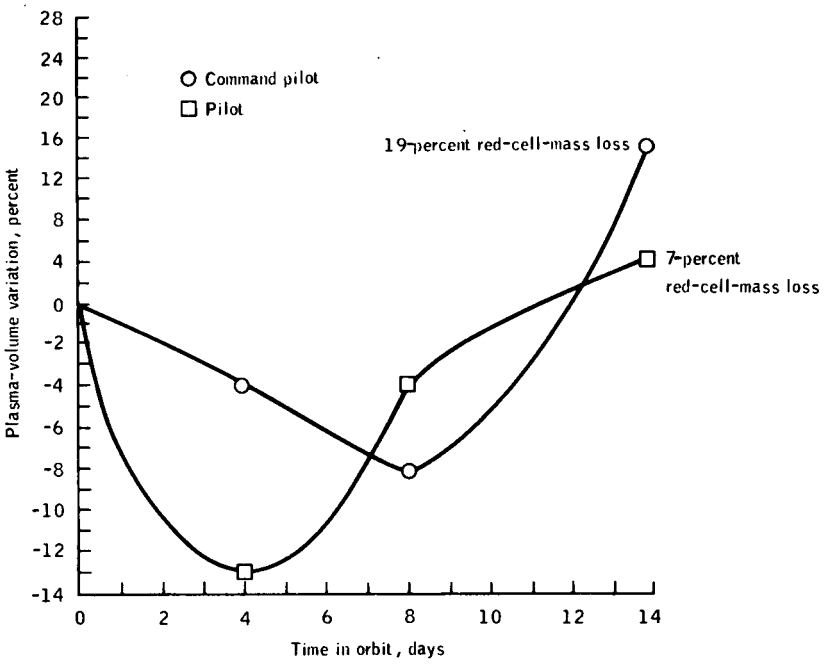
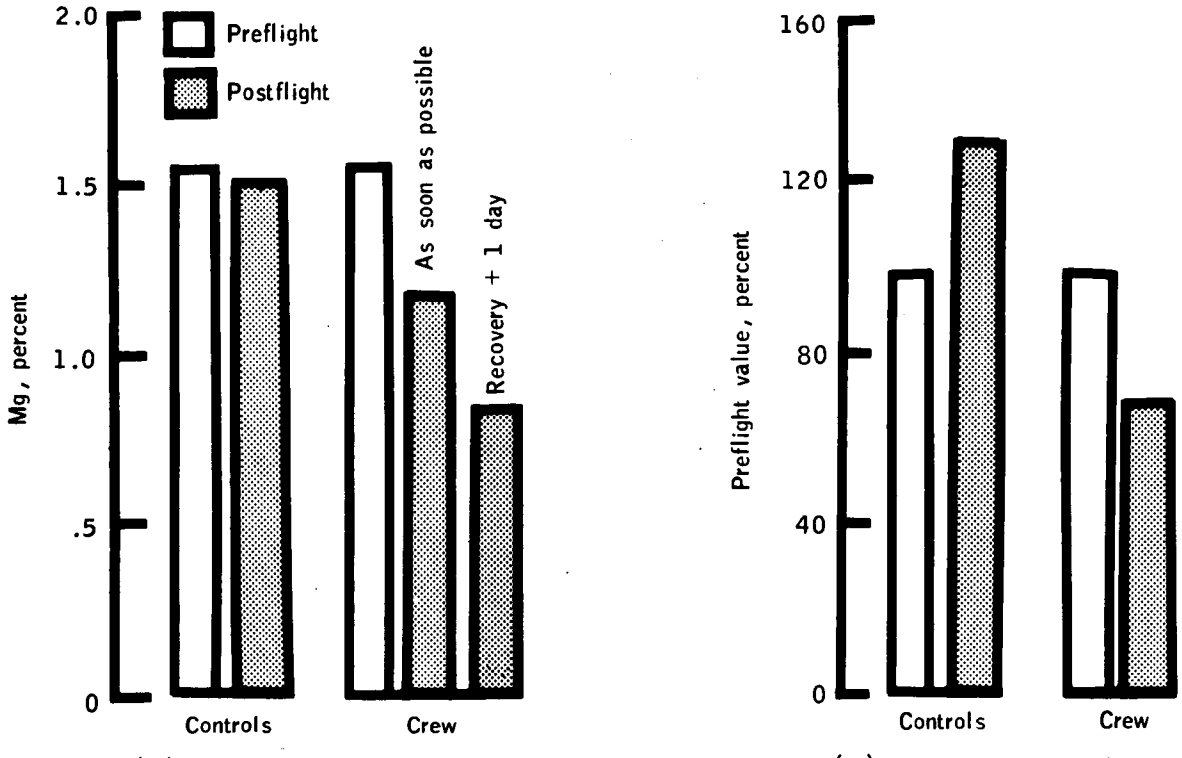


Figure 2-2.- Gemini Program plasma-volume data.



(a) Plasma vitamin E. (b) Plasma vitamin A.

Figure 2-3.- Plasma vitamin E and plasma vitamin A levels of Apollo 9 crew.

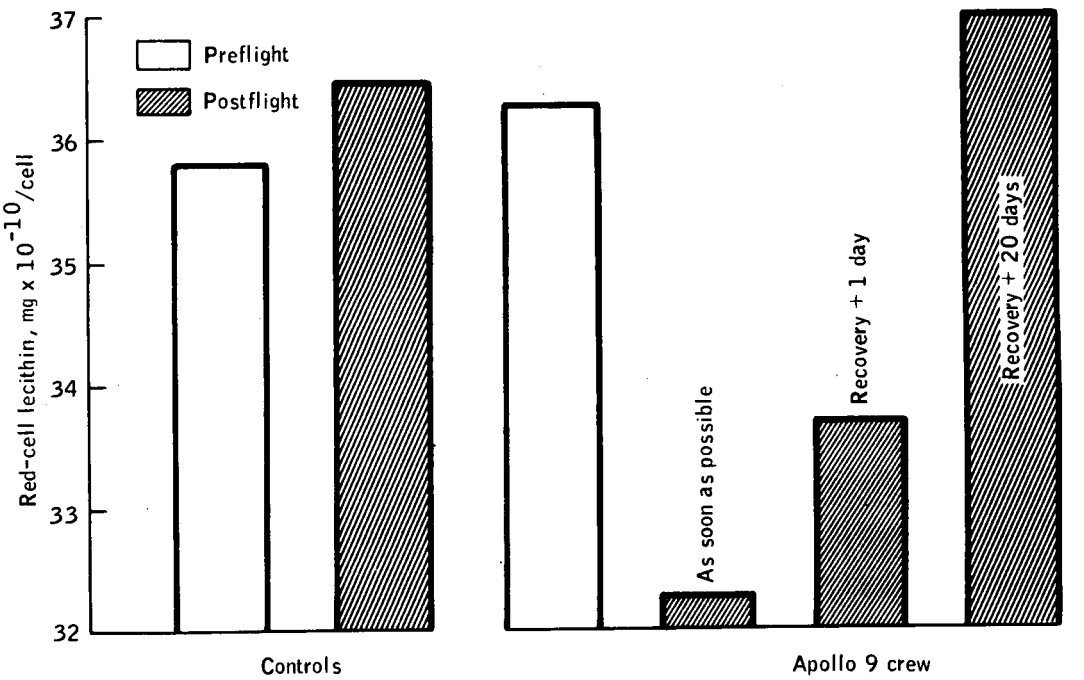


Figure 2-4.- Red-cell lecithin level of Apollo 9 crew.

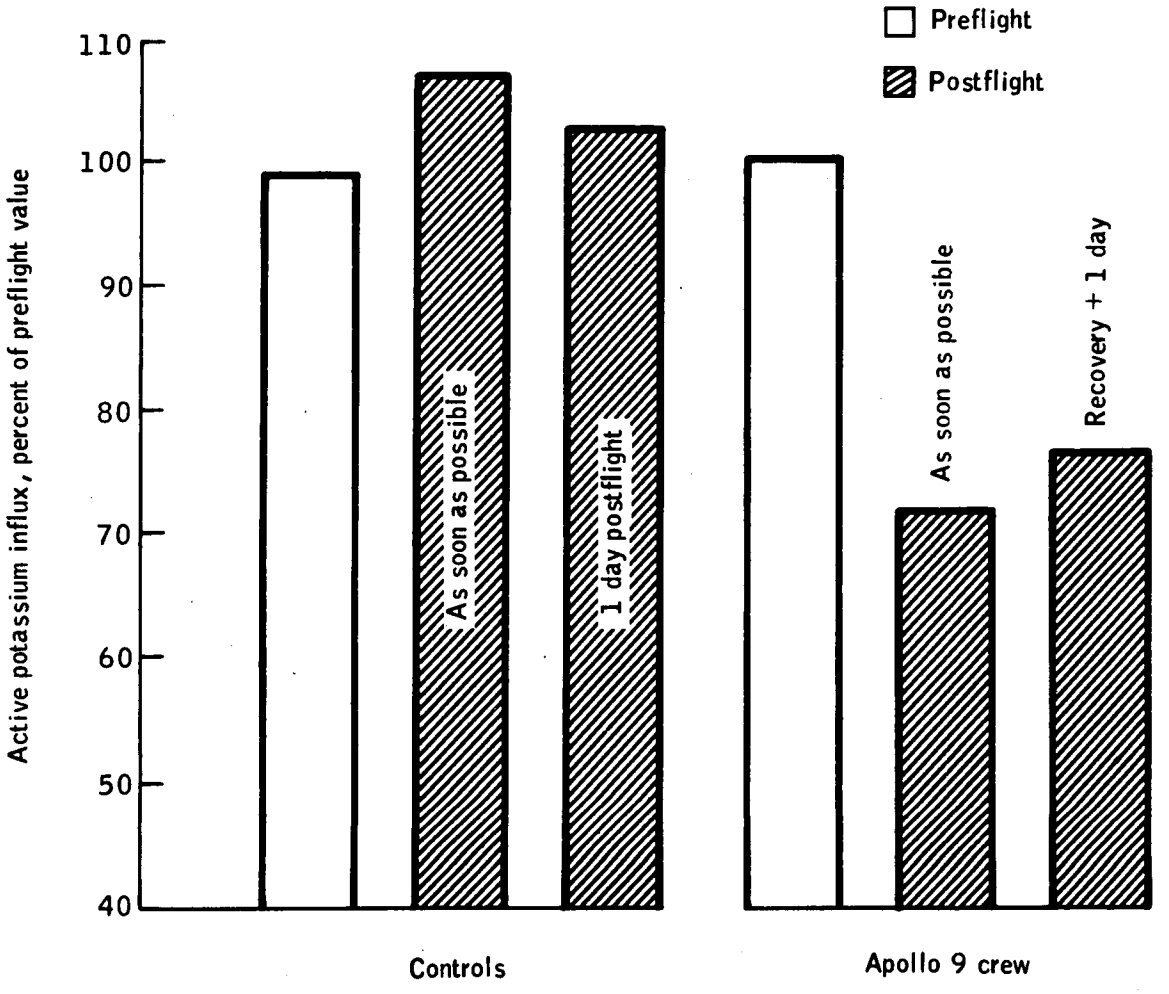


Figure 2-5.- Active potassium influx of red blood cells of Apollo 9 crew.

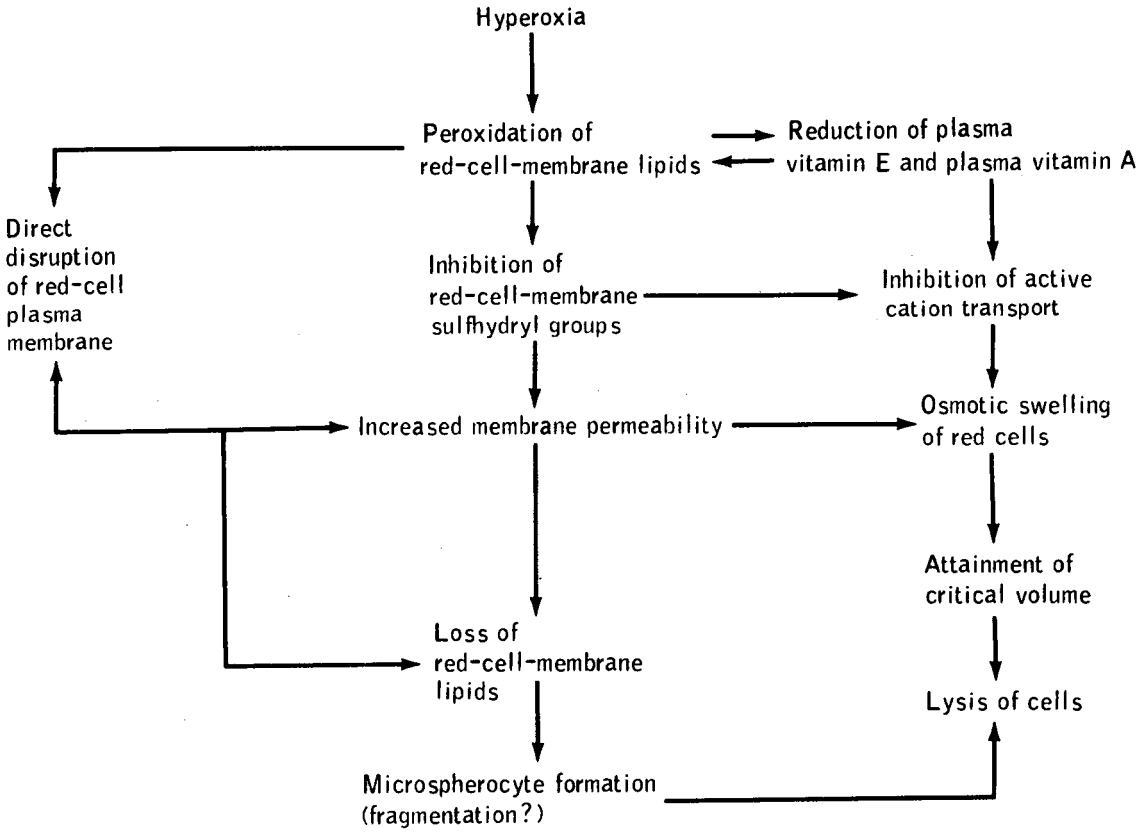


Figure 2-6.- "Best fit" hypothesis concerning the event sequence in red-cell hemolysis by hyperoxia.

3. REVIEW OF ENDOCRINE RESULTS:

PROJECT MERCURY, GEMINI PROGRAM, AND APOLLO PROGRAM

By Carolyn S. Leach, Ph. D.*

PROJECT MERCURY

When Project Mercury was in the planning stages, the hazards and stresses of space flight were unknown quantities. Because the adrenal gland is evidently one of the body's major responders to stress, it was natural to include the responses of this gland among the physiological parameters to be evaluated.

Considering typical data collected on Mercury crewmen, (fig. 3-1), one can observe changes in the plasma 17-hydroxycorticosteroid concentration. It is of interest to note the comparison of the changes observed after flight to changes after routine exercise in the preflight period.

Dr. Weil-Malherbe studied the catecholamine excretion rates of the Mercury crews. These measurements were made during periods free from imposed stress, during stressful training procedures, and finally during and after space flight. Increased excretion rates of substances measured, indicating an activation of the sympathoadrenal system, were observed after training procedures on some occasions; but, on others, the same procedures were tolerated without a significant sympathoadrenal response. In the same way, space flight induced increased excretion rates in some pilots but not in others. In the cases studied, the substance that was most frequently excreted at significantly increased rate was epinephrine (ref. 3-1).

At the completion of Project Mercury, endocrine data indicated that space flight evoked changes in catecholamines and 17-hydroxycorticosteroids. In both areas, changes caused by the mission were equal to or less than changes observed after training procedures.

Project Mercury had demonstrated impressively that man could survive and function ably as a pilot/engineer/experimenter in the space

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environment for periods of as long as 34 hours without undesirable reactions or detriment to normal body functions. Other medical knowledge gained included the fact that no evidence of abnormal sensory or psychological response was observed during an orbital space flight lasting as long as 1.5 days. However, after missions of 9- and 34-hour durations, an orthostatic rise in heart rate and decrease in blood pressure were noted that persisted for 7 to 19 hours after landing. The changes after the 34-hour mission were of greater magnitude than those after the 9-hour mission, but all changes disappeared in a similar time interval in both cases. The implications of this hemodynamic response obviously would require serious study before longer space missions. No other clearly significant changes were found in the comprehensive preflight and postflight physiological examinations.

Basic questions concerning space medicine remained unresolved. Certainly one of the most outstanding questions concerned the possible effects of prolonged weightlessness and combined stresses upon the astronauts.

GEMINI PROGRAM

The biomedical program that evolved for the Gemini Program was designed with the intent of determining, by the analysis of biological fluids, the metabolic cost of manned space flight. The results of these biochemical analyses were used as indications of the physiological status of the astronaut. Where changes were found to occur, efforts were made to understand the causative mechanisms and to assess their significance relative to the space flight. The measurements can be divided into three parts. The first part consists of preflight collection of urine samples and whole blood from each crewman to establish base-line values for the individual; the second portion of the measurements is the assessment of the physiological status of the crewmen using analysis of urine samples collected inflight; and urine and blood samples collected immediately postflight are used for the final portion. Within this context, a series of biochemical determinations was performed, consisting essentially of fluid and electrolyte balance, hormonal studies, and correlation of the two (ref. 3-2).

The data collected on the Gemini V, VI, and VII missions are shown in figure 3-2. The preflight values are considered to be 100 percent and the postflight values are compared with the preflight. The postflight changes that have been attributed to the space-flight environment are easily observed. To date, the Gemini VII mission has been the longest U.S. space flight. This mission was also the only one on which complete collection of inflight urine samples has been attempted.

An in-depth calcium-balance study was conducted by Dr. G. Donald Whedon, with endocrine parameters considered by Dr. Harry S. Lipscomb (ref. 3-3). The major endocrine changes reported include inflight catecholamine increases that continued for 24 hours after the missions. The 17-hydroxycorticosteroid values were low during the 14-day orbital mission but were elevated in the first day after recovery. These findings are discussed in detail in section 10 of this report. Other endocrine and electrolyte data were collected before and after the Gemini VIII and IX missions (ref. 3-4). The results are similar to those observed on the Gemini VII mission.

APOLLO PROGRAM

The endocrine program developed to support the medical requirements for the Apollo missions was constrained by the limitation to preflight and postflight testing, so no inflight samples have been returned for analysis.

To consider which hormones warranted thorough study in relation to man in the space-flight environment, the endocrine program was established and is currently being implemented in two specific areas under the broad title Neuroendocrine Control of the Adaptation to Space Flight. The two areas of study are (1) the quantification of stress by the measurement of adrenal cortical and medullary hormones with the consideration of the effect of time and pituitary stimulation on these adrenal secretions and (2) the endocrine control of fluid and electrolyte balance.

Adrenal Response

First, with regard to adrenal secretions, one can observe that the catecholamine responses (figs. 3-3 and 3-4) during the missions considered are much like those noted in the Mercury and Gemini data. The epinephrine and norepinephrine concentrations are generally increased in the first 24 hours after the mission. It also appears, though the sampling rate is still low for statistical confirmation, that epinephrine is more sensitive to the stress of space flight. The plasma- and urinary-hydrocortisone data (figs. 3-5 and 3-6) indicate a striking and unexplained phenomenon in the immediate-postflight sampling. It is evident that the plasma-hydrocortisone values are below preflight values in the blood sample taken within 3 hours after splashdown. However, the integrated urine sample, collected the first 24 hours after recovery, shows a general increase in the excretion of hydrocortisone. These results were not anticipated because the hydrocortisone was expected to increase after space flight. After this finding was noted, adrenocorticotrophic hormone (ACTH) was measured in the immediate-postflight blood sample taken

from the Apollo 8 crewmen. The results (table 3-I) did not indicate any significant change from preflight values. However, it is necessary to consider the extremely short biologic half-life of ACTH; and the possibility exists that this blood sample was not taken in time to observe a change from normal. In an attempt to interpret this finding, the possibility of time influence upon adrenal activity was considered; that is, if the preflight blood samples were taken in the morning, the values could be higher than samples taken in the afternoon. However, the launch and recovery times were compared (table 3-II) and this factor does not appear to be the reason for the decreased postflight plasma-hydrocortisone value. Other possibilities are now being considered.

Fluid and Electrolyte Balance

The second major area being studied in the endocrine program is the control of fluid and electrolyte balance by the endocrine system. This part of the program was established for two reasons. First, experience gained from Mercury, Gemini, and early Apollo missions indicated dynamic postflight changes in hormones active in fluid and electrolyte control. The second reason was the physical and clinical characteristics of the crewmen returning from space flight. For these reasons, the laboratory personnel have considered the profiles of fluid and electrolyte balance as related to the loss in body weight that consistently occurs during space flight and have correlated these weight changes to alterations in body-fluid distribution and the electrolyte compartments.

Body-fluid losses in crewmen on United States and Russian manned space flights have been consistently inferred by a 2.5- to 5-percent decrease in postflight body weight (table 3-III). This loss averages 6 lb/crewman with over 50 percent of the weight regained within the first 24 hours after splashdown. On those missions on which plasma volume was measured, a postflight decrease has generally been observed.

With these body weight changes used as indicators of fluid changes, a study was undertaken to consider the cations and anions that serve critical roles in the homeostatic regulation of blood volume. The urinary- and plasma-electrolyte values demonstrated significant and constant changes in some specific ions. Plasma sodium and potassium values demonstrate a decreasing trend in the first postflight sample with no real consistency in the plasma chloride. The urine samples collected the first 24 hours after recovery provide data demonstrating the retention of sodium and chloride. To aid in the understanding of water and electrolyte balance and renal function, the hormone vasopressin (the antidiuretic hormone (ADH) from the posterior lobe of the pituitary) and the adrenal mineralcorticoid (aldosterone) were measured in the urine.

The urinary ADH values (fig. 3-7) demonstrate an increase in the immediate postrecovery 24-hour sample for most of the crewmen. These numbers correlate very well with the observed weight loss of these crewmen, indicating that the weight loss is probably fluid related. The urinary-aldosterone values (figs. 3-8 and 3-9) have been generally increased. It is of interest to note that the increase does not occur until the second postflight day on some of the crewmen studied.

To pursue the investigation one more step, the plasma was examined for angiotensin I — a direct indication of renin activity — to ascertain the role of the kidneys in the aldosterone response. These data (fig. 3-10) demonstrate a significant increase in most of the crewmen. These very significant values indicate that the kidneys are responsive to the change in effective blood volume being presented to them.

After these data have been examined and the fact has been taken into account that no clinical conditions associated with space flight have occurred that could be attributed to the fluid, electrolyte, and endocrine changes, a schema proposing an adaptation to the weightlessness environment (ref. 3-5) is presented (fig. 3-11).

It is believed that, while the crewman is in a weightless state, his blood volume is evidently redistributed particularly from the lower extremities into the abdomen and thorax and that this situation initiates a diuresis that has been described by Gauer and Henry (ref. 3-6). This diuresis is checked by an aldosterone response that would cause a sodium retention and a selective potassium loss — such as were observed in the Gemini VII crewmen. Because these changes were observed on the only mission on which the opportunity for investigation existed, the proposed schema is not all just postulated. After this adaptation, the crewmen are in flight in a new steady-state condition in which a lower total-body-water content and a lower total-body-potassium content are experienced. If this situation indeed reaches significant proportions the crewmen's bodies then exchange hydrogen ions to prevent further K^+ loss, resulting in an intracellular acidosis. This situation, of course, would result in real compensations that do not appear in the proposed schema, but that would override the immediate respiratory response. The point is that a decrease in total body potassium occurs that sets the stage for intracellular acidosis and extracellular hypokalemic alkalosis. As much as a 20-percent postflight decrease in total body potassium has been measured, by low-level total-body-counter monitoring of the total body potassium-40. Also, the returning crewmen's bodies retain potassium and sodium fluids so that these particular aspects of the schema are evidently appropriate. It is interesting to note that some positive feedback loops are proposed in the schema. Once this chain of events begins, a decrease in total body potassium follows; of course, some loss

of potassium in the urine results, leading to a positive feedback system that can be dangerous if the body is not provided with both potassium and chloride ions.

CONCLUSION

It should be emphasized that the problem of endocrine control of fluid and electrolyte balance associated with space flight is just now being considered in depth. Although it is not believed that the changes observed constitute any immediate danger to the crewmen, it is thought that this adaptation to a weightless state costs energy and uses reserves, a situation that places homeostatic mechanisms at the edge of the safety margin; furthermore, a severe stress beyond this weightless state would prove a difficult challenge for these control mechanisms.

The second area of endocrine investigation (the quantification of stress) has been equally productive. The measurement of adrenal hormone activity has provided assessment of each crewman's response to the space-flight environment. However, more data are needed for a prediction of postflight response on the basis of preflight values; and, only when samples are taken during the flight, will this correlation be complete. These examinations will not be possible until the earth-orbital Skylab missions, currently scheduled for 1973. Until that time, the pieces of this puzzle will be fitted together to yield immediate understanding as well as to ensure all necessary parameters will be considered during the inflight Skylab experiment.

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TABLE 3-I.- ADRENOCORTICOTROPHIC-HORMONE CONCENTRATIONS — APOLLO 8 MISSION

[Normal range is 10 to 40 pg/ml]

Subject	Concentration, pg/ml, at -			
	14 days preflight	5 days preflight	As soon as possible postflight	6 days postflight
Commander	38	35	27	22
Command module pilot	22	40	30	25
Lunar module pilot	30	27	18	33
Control	25	35	20	22

TABLE 3-II.- BLOOD-SAMPLE-COLLECTION TIMES

Apollo mission	Preflight (Kennedy Space Center) collection period, e.s.t., hr:min	Immediately postflight (recovery ship) collection period, e.s.t., hr:min
8	7:30 to 8:30 a.m.	12:00 p.m.
9	7:30 to 8:30 a.m.	1:30 p.m.
10	7:30 to 8:30 a.m.	2:00 p.m.
11	7:30 to 8:30 a.m.	2:00 p.m.
12	7:30 to 8:30 a.m.	5:00 p.m.
13	7:30 to 8:30 a.m.	3:00 p.m.

TABLE 3-III.- WEIGHT LOSS DURING SPACE FLIGHT

Crewman	Mission	Duration, days	Loss of weight (a)		Crewman	Mission	Duration, days	Loss of weight (a)	
			Absolute	Percent				Absolute	Percent
Gagarin	Vostok 1	0.1	0.1	0.7	Cernan	Gemini IX	3	-8.0	4.6
Titov	Vostok 2	1	3.96	2.9	Young	Gemini X	3	-4.0	2.8
Nikolayev	Vostok 3	4	3.96	2.6	Collins	Gemini X	3	-7.5	4.6
Popovich	Vostok 4	3	4.62	2.8	Conrad	Gemini XI	3	-2.7	1.8
Bykovskiy	Vostok 5	5	5.28	3.6	Gordon	Gemini XI	3	.0	--
Tereshkova	Vostok 6	3	4.12	3.3	Lovell	Gemini XII	4	-9.7	5.7
Komarov	Voskhod I	1	4.18	2.7	Aldrin	Gemini XII	4	-4.7	2.8
Feoktistov	Voskhod I	1	6.38	4.0	Schirra	Apollo 7	11	-6.3	3.2
Yegerov	Voskhod I	1	6.60	3.9	Eisele	Apollo 7	11	-10.0	6.4
Belyayev	Voskhod II	1	2.2	2.0	Cunningham	Apollo 7	11	-8.0	5.1
Leonov	Voskhod II	1	1.98	2.0	Borman	Apollo 8	8	-8.75	5.2
Glenn	Mercury 6	.2	5.28	3.1	Lovell	Apollo 8	8	-7.8	4.5
Carpenter	Mercury 7	.2	5.94	3.9	Anders	Apollo 8	8	-4.0	2.8
Schirra	Mercury 8	.4	4.0	2.8	McDivitt	Apollo 9	10	-5.25	3.2
Cooper	Mercury 9	1.4	7.7	5.2	Scott	Apollo 9	10	-5.75	3.3
Grissom	Gemini III	.2	-3.0	1.7	Schweickart	Apollo 9	10	-6.12	3.8
Young	Gemini III	.2	-1.5	2.1	Stafford	Apollo 10	8	-2.0	1.2
McDivitt	Gemini IV	4	-4.5	2.9	Young	Apollo 10	8	-5.75	3.5
White	Gemini IV	4	-8.5	4.9	Cernan	Apollo 10	8	-9.5	5.5
Cooper	Gemini V	8	-7.5	4.9	Armstrong	Apollo 11	8	-7.5	4.4
Conrad	Gemini V	8	-7.2	5.5	Collins	Apollo 11	8	-6.0	4.2
Schirra	Gemini VI	1.1	-2.6	1.3	Aldrin	Apollo 11	8	-1.25	.8
Stafford	Gemini VI	1.1	-10.0	4.9	Conrad	Apollo 12	10	-5.0	3.3
Borman	Gemini VII	14	-10.0	6.2	Gordon	Apollo 12	10	-7.0	4.5
Lovell	Gemini VII	14	-6.0	3.7	Bean	Apollo 12	10	-14.0	9.1
Armstrong	Gemini VIII	.5	--	--	Lovell	Apollo 13	6	-15.0	8.4
Scott	Gemini VIII	.5	--	--	Swigert	Apollo 13	6	-10.0	5.1
Stafford	Gemini IX	3	-2.0	1.2	Haise	Apollo 13	6	-16.0	9.7

[a]Preflight weight compared with immediately measured postflight weight.

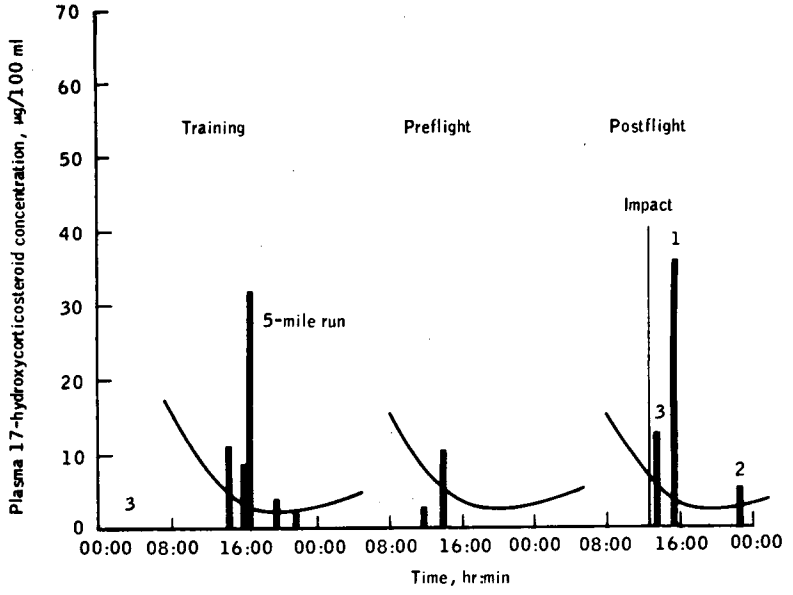

Figure 3-1.- Biochemical data — Project Mercury.

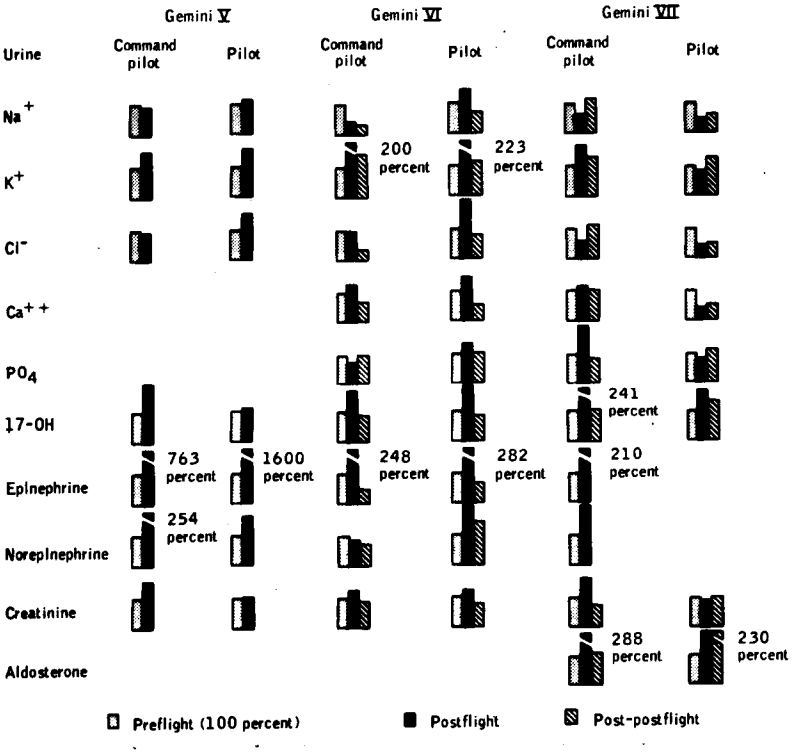

Figure 3-2.- Biochemical data — Gemini V, VI, and VII missions.

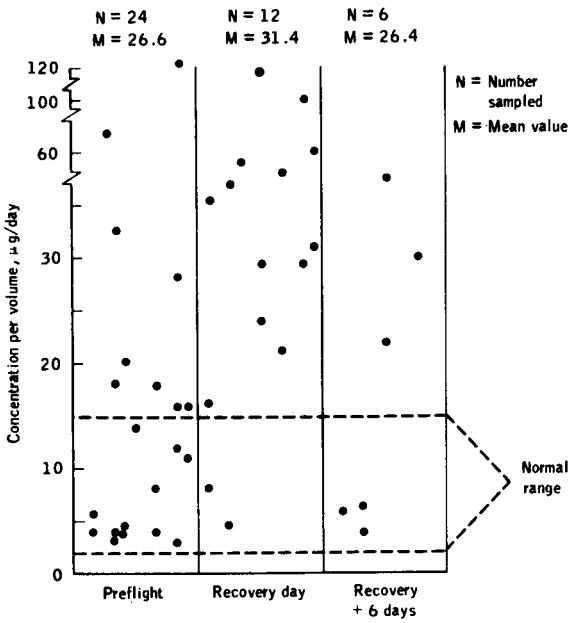

Figure 3-3.- Urinary-epinephrine concentrations — Apollo 8, 9, 10, 11, and 13 missions.

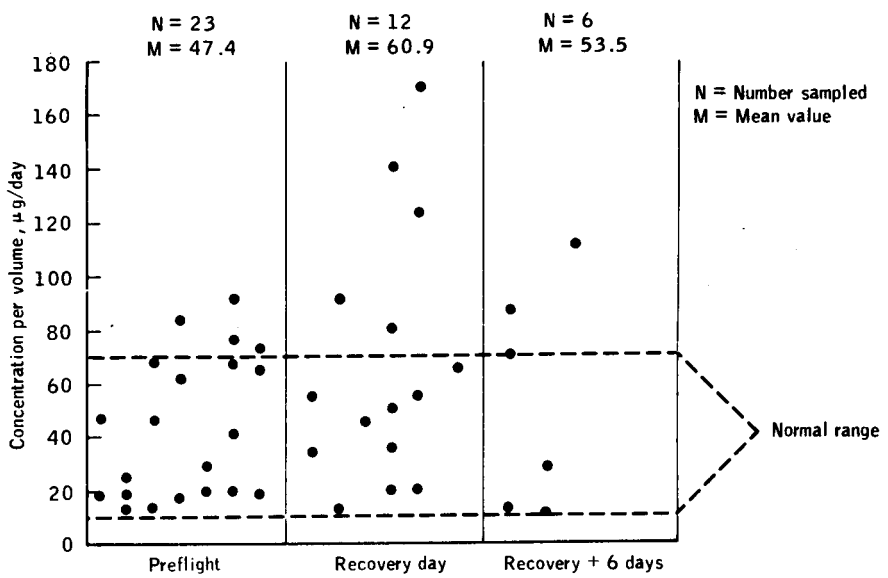

Figure 3-4.- Urinary-norepinephrine concentrations — Apollo 8, 9, 10, 11, and 13 missions.

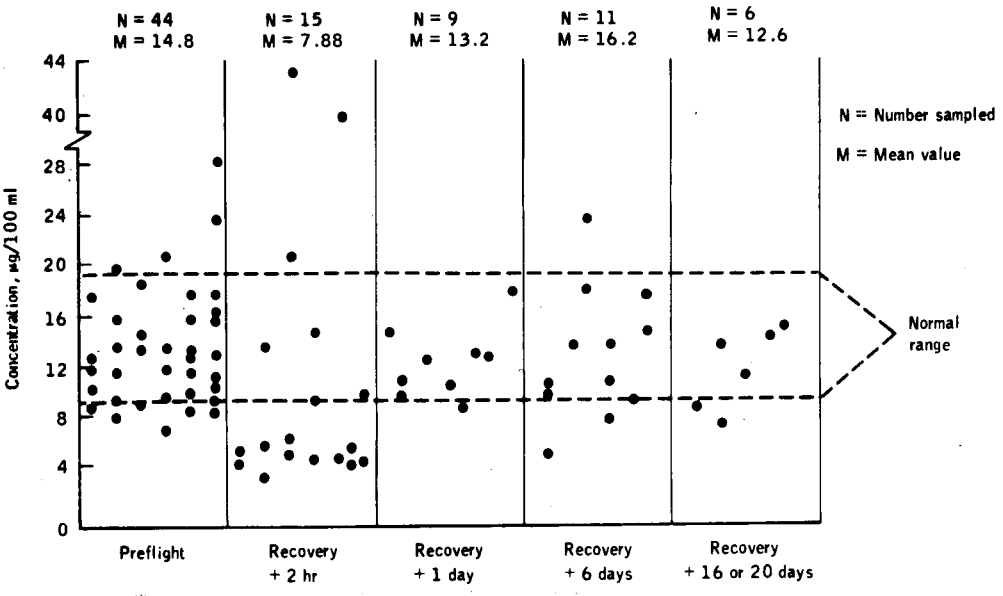

Figure 3-5.- Plasma-hydrocortisone concentrations — Apollo 8, 9, 10, 11, 12 and 13 missions.

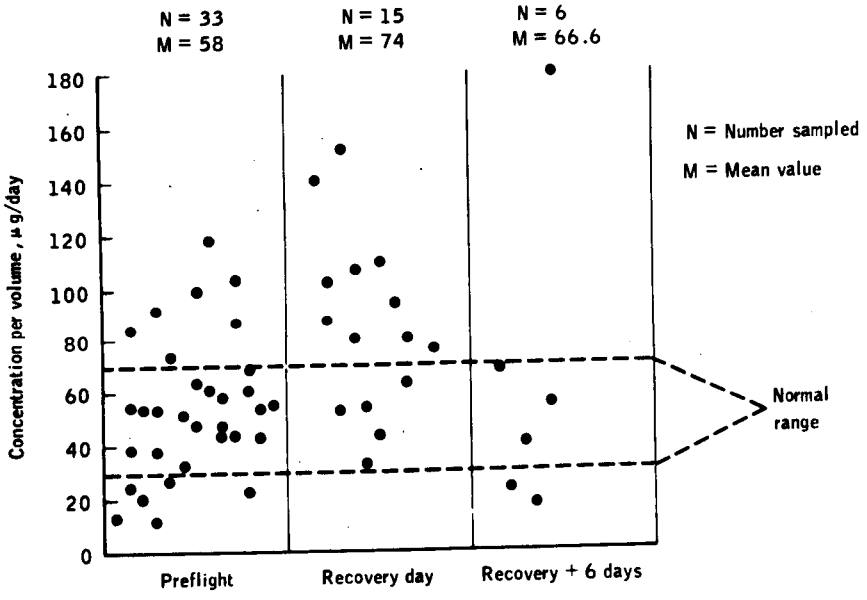

Figure 3-6.- Urinary-hydrocortisone concentrations — Apollo 8, 9, 10, 11, and 13 missions.

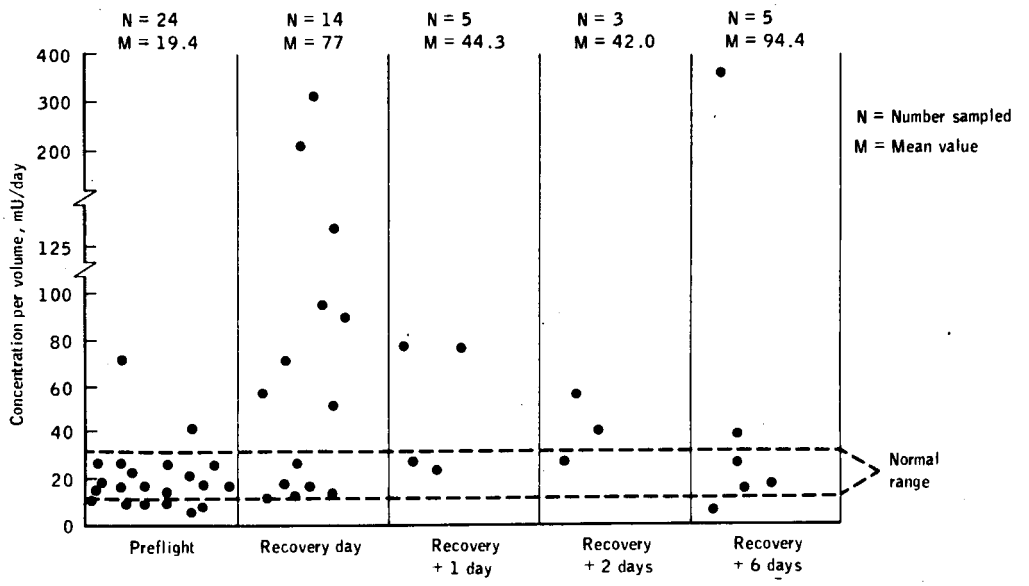

Figure 3-7.- Urinary-antidiuretic-hormone concentrations — Apollo 8, 10, 11, 12, and 13 missions.

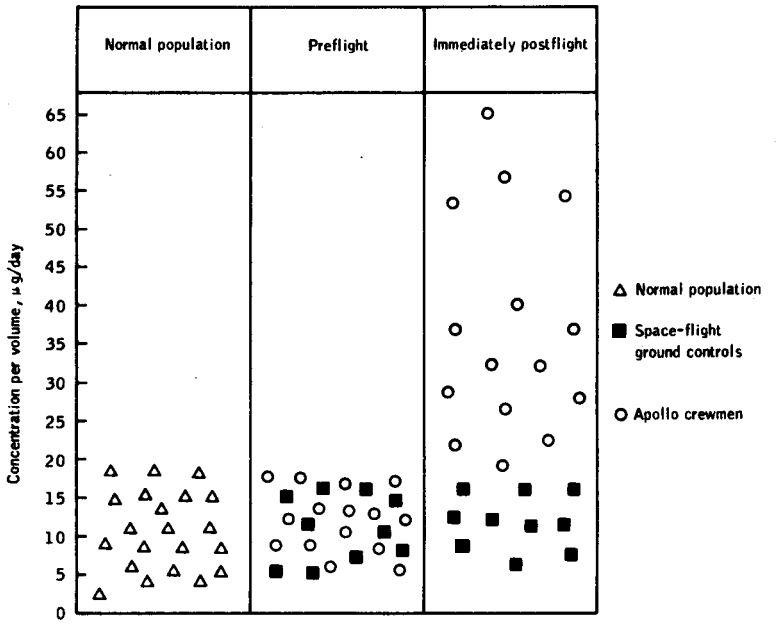

Figure 3-8.- Urinary-aldosterone concentrations — controls and Apollo crewmen.

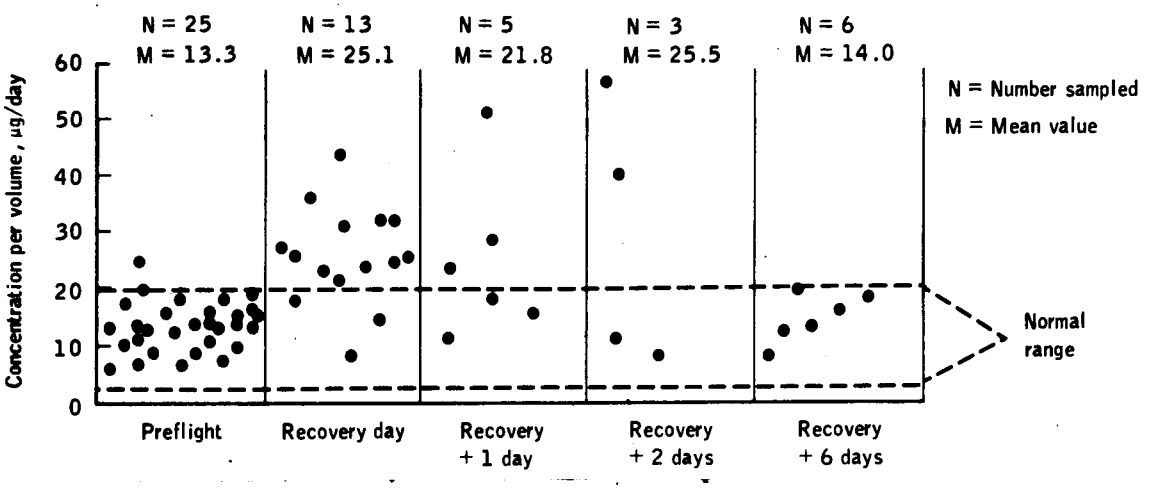

Figure 3-9.- Urinary-aldosterone concentrations — Apollo 8, 9, 10, 11, and 12 missions.

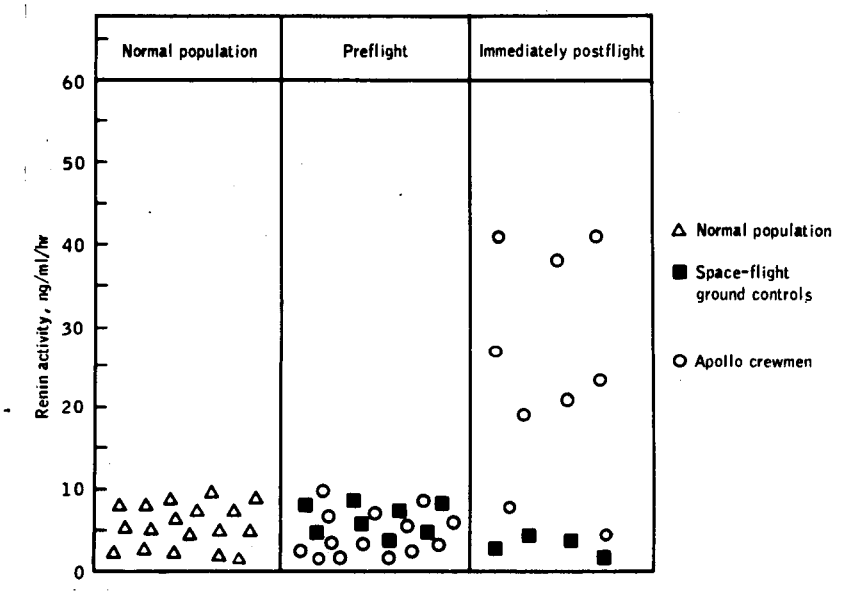

Figure 3-10.- Plasma-renin activity — controls and Apollo crewmen.

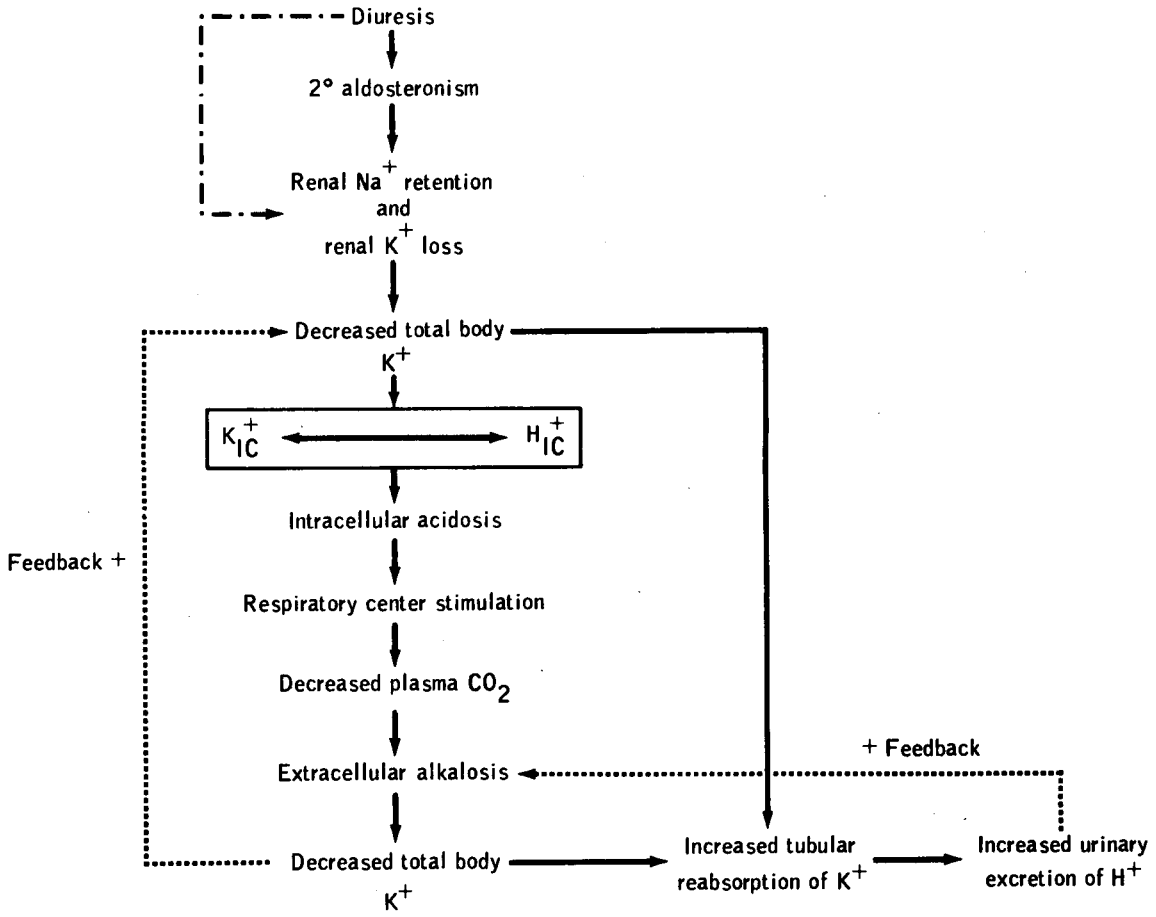

Figure 3-11.- Schema of proposed adaptation to weightless environment.

4. REVIEW OF METABOLIC AND ENDOCRINE STUDIES ON THE GEMINI VII MISSION

By Harry S. Lipscomb, M.D., F.A.C.P.*

INTRODUCTION

As the Apollo Program moves into the final phases and preparation is made for long-term, earth-orbital missions, it may be worthwhile to review some of the earlier studies derived from the Gemini Program. It was the author's good fortune to be involved in the metabolic studies of a number of the Gemini flights. The 14-day Gemini VII earth-orbital mission was the most completely documented (ref. 4-1).

This unique study was planned within the rigorous constraints of the technical characteristics of the mission itself. For this reason, it was a formidable research task to ensure that adequate collection of samples was obtained and that adequate preflight and postflight followup studies were conducted properly.

The primary goal of these studies was to measure changes, if any, that may have been produced by the period of zero gravity in space on total body metabolism related both to the musculoskeletal and endocrine systems. The crucial point in the validity of the data derived from the Gemini VII mission was the necessity to have correct inflight urinary-excretion volumes. Unaltered 24-hour renal clearance and urinary excretion of creatinine had to be assumed. It was necessary to ascribe the low and variable urinary creatinine values to the known incomplete and variable urine collections in flight. It was impossible to ascribe this lower urinary creatinine value in flight to muscle wastage because, when subjects undergo several weeks of extreme immobilization and bedrest in casts, their urinary creatinine has not declined. The findings of the Gemini VII mission are divided into several sections.

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DISCUSSION

Calcium Metabolism

Increases of 23 and 9 percent were observed, respectively, in mean urinary calcium excretion in the two crewmen. It has been described earlier that immobilization by disease or in experimental studies leads to increased bone resorption leading to hypercalciuria and eventual osteoporosis. These earlier bedrest studies clearly showed a much more striking hypercalciuria than that observed in the Gemini VII crewmen.

The somewhat lower levels of hypercalciuria in manned space flight, as contrasted with those seen experimentally in bedrest studies, were indicative of several possibilities. First, it was evident that use of the bungee and other moderate physical activity of the mission itself could have accounted for the somewhat lower levels of calcium excretion. The gaseous atmosphere of the spacecraft (100 percent oxygen at 260 torr) has been shown to increase bone resorption in the experimental environment, and it was believed that hyperoxia might contribute further to losses of calcium. Conversely, high altitude has been demonstrated to decrease or suppress losses of calcium during bedrest.

It is of interest that the urinary excretion of calcium did not change significantly during the first 7 days of space flight in either crewman; but, starting on the eighth day, a definite increase occurred for the command pilot and persisted during the 4 days of postflight observation. Dermal losses of calcium were low for both crewmen during flight.

Phosphate

A significant increase in urinary phosphate excretion was noted over the first 9 days of space flight. Thereafter, despite relatively constant dietary intake, urinary excretion of phosphate dropped to near control levels.

Sulfate

Urinary sulfate excretion did not change significantly during space flight. Sulfate excretion data tend to resemble those of nitrogen.

Nitrogen

Urinary nitrogen decreased in both crewmen during flight and returned to preflight values during the postflight phase. Dietary nitrogen

intake was significantly less during the flight, with the result that nitrogen balance became negative during the flight phase.

Magnesium

Urinary excretion of magnesium did not change during the first week of space flight; but, in the command pilot, significantly increased amounts of magnesium were excreted in the second week, as noted for calcium excretion. Toward the end of the mission and persisting through 4 days of postflight study, urinary magnesium excretion decreased dramatically. The copilot's pattern was similar but changes were not significant.

Potassium

Potassium excretion varied strikingly between the two crewmen. The command pilot showed an initial decrease in urinary potassium in flight in the presence of a marked decrease in dietary potassium. During the second week of flight, urinary potassium rose; but, immediately after the flight, potassium excretion fell to preflight values as the dietary intake was increased. On the other hand, the copilot demonstrated only a slight decrease in urinary potassium in the first week of flight despite a marked restriction in intake. During the second week, excretion decreased further and rose to preflight values during the recovery phase.

Sodium and Chloride

Again, different patterns were observed between the crewmen. Despite a slight decrease in dietary sodium, the command pilot experienced increased sodium excretion during the first week of flight, a return to control values during the second week, and significant retention in the early postflight period. Conversely, the copilot demonstrated no change in sodium excretion during the first part of the flight, an increase thereafter, but then, similar to the command pilot, marked retention in the postflight period.

Hormone Excretion

In the copilot, epinephrine excretion and, more variably, norepinephrine excretion were greatest on the 2 days of the greatest predicted stress — the day of lift-off and the day of splashdown. The command pilot catecholamine excretion approximated this pattern, but the values were not significantly different from the control, preflight phase.

Excretion of 17-hydroxycorticosteroids was low in both crewmen throughout the entire flight. In both subjects, this measurement was elevated only on the day of splashdown. The few values for urinary aldosterone obtained were elevated during and immediately after the flight.

CONCLUSIONS

In view of the numerous influences in space flight — notably 100-percent-oxygen atmosphere, one-third atmospheric pressure, and relatively uncontrolled but strikingly limited physical activity, in addition to weightlessness — the metabolic effects of manned space flight in the weightless condition, as such, were not determined definitively. The various metabolic changes observed represent the net effect of several different (both concurrent and counteracting) factors, predominantly physical in nature. Within the broad limits of precision of this first metabolic study in space, changes in mineral and hormonal balance were sufficiently modest to support, from the metabolic point of view, the decisions that a voyage to and return from the moon would be medically safe, because the time required would be no more and, in fact, less than the duration of the Gemini VII mission. In much longer missions, however, the necessity for additional metabolic observations is evident.

In particular, it is believed that most careful scrutiny must be given to the insistence on exercise on a regular basis as an integral part of space flight to prevent predicted calcium losses in urine and the subsequent destruction of bone. Equally important, the changes observed in red-blood-cell mass (described by other conference attendees) — most reasonably associated with the atmospheric conditions of flight — and the changes in blood-pressure maintenance and regulation will require most scrupulous attention. Finally, early highly suggestive evidence exists to support the contention that glomerular filtration and renal function (as it relates to aldosterone and steroid secretion) requires more careful study.

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5. JOINT AMES RESEARCH CENTER/MANNED SPACECRAFT CENTER STUDY: DIURNAL VARIATION IN ADRENOCORTICAL AND THYROID FUNCTION DURING PROLONGED BEDREST

By Joan Vernikos-Danellis, Ph. D.*

INTRODUCTION

At the NASA Ames Research Center, the emphasis in the endocrine research program that will be discussed has been mostly on the factors affecting the endocrine system, particularly the hypothalamic-pituitary-adrenal system and the stress response. The program has involved such various aspects as the study of the mechanisms mediating the secretion of corticotropin-releasing hormone and adrenocorticotrophic hormone (ACTH), feedback mechanisms, central and peripheral autonomic mechanisms, drugs, psychological and environmental variables that affect the stress response, and the development of methods for the bioassay of these hormones in blood and other tissues. Most of this work has been done in animals, primarily the rat, and to a smaller extent in man.

More directly space-related work has involved entirely the use of simulation of weightlessness by bedrest in man or extrapolation by exposure of rats to increased g-loads by chronic centrifugation and comparison with exposure to other chronic stress stimuli.

What has become evident from the chronic studies in rats has been the transient or lack of apparent changes in "basal" levels of ACTH or corticosteroids (if sampled at a single point daily), although very marked effects were evident in the magnitude and direction of the response to an additional acute standardized stimulus (such as 1 minute ether), and these effects were dependent on the duration and intensity of exposure. It has been my contention, therefore, that, to assess the ability of the pituitary-adrenal system not only to maintain normal physiological function but also to respond to an added unexpected challenge at various points during chronic exposure to some alteration in the environment, it may be desirable (in addition to estimating basal levels) to impose tests of specific endocrine organ function.

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DISCUSSION

In an effort to collect preliminary information about changes in the endocrine system of man during prolonged exposure to hypokinesic conditions and to postural changes and confinement, cortisol, thyroxine, and tri-iodothyronine blood levels were determined during a 56-day bedrest study that was performed under contract by the Manned Spacecraft Center.

The circadian rhythm of the plasma adrenocortical hormone levels in humans has been described by several investigators and appears to be well established. On the other hand, the possibility of a diurnal variation in thyroid activity has been investigated using only indirect indices without definite conclusions being achieved. For instance, fluctuations in protein-bound iodine have been reported to run parallel to adrenal activity, whereas the iodine-131 uptake of the thyroid gland shows the opposite pattern with the peak between 7:30 and 11:30 p.m. (ref. 5-1).

Past bedrest studies in healthy human subjects have generally ignored any changes in the endocrine system with the exception of sporadic daily or even weekly urinary steroid-excretion measurements. Only the 1965 study of Cardus et al. (ref. 5-2), measuring the circadian rhythm of plasma levels of 17-hydroxycorticosteroids, reports no significant changes after 3 days of bedrest.

Eight healthy males, aged 20 to 40, were submitted to bedrest for 56 days on a 14-hour-light, 10-hour-dark regimen (lights on at 9:00 a.m.) and fed an Apollo diet. Four of these subjects exercised with an Exer-Genie three times daily throughout the experiment. Blood samples were drawn by repetitive venous punctures at hourly intervals for 48-hour periods before bedrest, at 10, 20, 30, 42, and 54 days after confinement to bed, and 10 days after the subjects had again been ambulatory. Plasma-free cortisol levels were determined by Murphy's competitive protein-binding radioassay and expressed as μg/100 ml plasma. Serum total thyroxine was expressed as μg/100 ml serum, and serum tri-iodothyronine was estimated by determining the binding capacity of serum to the hormone and expressed as relative percent uptake.

The relationship of the rhythm in circulating levels of hydrocortisone, thyroxine, and tri-iodothyronine in the eight subjects during their ambulatory prebedrest control period is shown in figure 5-1. All three hormones showed a significant diurnal fluctuation with maximal levels occurring at 7:30 a.m., anticipating lights on. In addition, thyroxine showed a secondary peak at 3:30 p.m. The amplitude of both thyroid-hormone rhythms was much smaller than that of the corticosteroid.

The plasma hydrocortisone rhythm at various intervals during the study in the four subjects that exercised and the four that did not is shown in figure 5-2. Bedrest had little effect on the circadian rhythmicity of this hormone. A significant fluctuation in plasma cortisol was evident with peak levels occurring around 7:30 a.m. throughout the experiment. However, progressive bedrest reduced the amplitude of the steroid rhythm and neither exercise nor the 10-day postbedrest ambulatory period prevented or corrected this reduction in amplitude. The occurrence of the peak in plasma cortisol was more variable in the exercised subjects.

The use of the summation dial to depict these results is illustrated in figure 5-3. The summation-dial method of describing nonstationary biological time series data was developed at the Ames Research Center by Hetherington, Rosenblatt, and Winget and emerged of necessity as a result of this bedrest study. Briefly, the curve best fitted the data is derived mathematically assuming a specified period (e.g., $\tau = 24$ hr). Each point on the curve represents the end of a vector with a certain magnitude and direction describing the phase of the rhythm for that day. The summation of these vectors or train of vectors produces the summation dial. The direction of the vector train is the hour of the day at which estimated peak activity occurred. The length of the vector indicates the amplitude of the rhythm. In this instance, it is shown that the exercised group of subjects peaked about 1 hour before the nonexercised.

In contrast to the remarkable stability of the plasma hydrocortisone rhythm, both thyroid hormones showed very unstable rhythms during the bedrest period with a return to original rhythmicity at the postbedrest collection period. This instability of the thyroxine rhythm is shown in figure 5-4; that of the tri-iodothyronine rhythm, in figure 5-5. Exercise did not appear to increase the stability of these rhythms.

Correlations over time between physiological parameters may be studied by use of the vector-difference dial that quantifies the angle between the summation dials of these two parameters. Comparison of cortisol with tri-iodothyronine rhythms in nonexercised subjects (fig. 5-6) throughout the study shows perfect correlation in the occurrence of the time of peak of the two rhythms for the prebedrest period and the first part of the study. A rephasing occurred about the 20th day of bedrest with the result that the hydrocortisone peak led the tri-iodothyronine by 6 hours. Additional rephasing occurred at about the 42nd day with the peak in tri-iodothyronine occurring 12 hours before that of plasma hydrocortisone. However, 10 days after the bedrest period, the thyroid and hydrocortisone rhythms again showed perfect correlation.

To illustrate the effect of continuous bedrest and of exercise on the mean circulating levels of these three hormones, the results are expressed somewhat differently in the final three figures.

The mean plasma cortisol per 48-hour sampling period (solid line) in the exercised and nonexercised subjects is shown in figure 5-7. The circles and connecting dashed lines in this and the next two figures represent the mean maximal and minimal concentrations (or amplitudes) that occurred during that period, irrespective of the hour of day that they occurred. In the nonexercised subjects, the results show a reduction in the mean daily cortisol level and a marked reduction in the daily range. The exercised subjects showed a transient rise in the mean daily output and in the daily range between the 10th and 14th day of bedrest, but this rise was not maintained and a reduction in amplitude followed as in the nonexercised subjects. It is important to note that the amplitude is still reduced at the end of the 10-day postbedrest ambulatory activity.

In a similar fashion, the changes in circulating thyroxine are shown in figure 5-8. Both exercised and nonexercised subjects show similar patterns. The only effect of bedrest appeared to be a transient increase in the mean daily concentration during the first 10 days with no change in amplitude throughout the experiment. However, in both groups of subjects, a dramatic increase in mean thyroxine levels was noted when the subjects got out of bed.

On the other hand, as shown in figure 5-9, the concentration of triiodothyronine markedly increased during the first few days of the study and remained elevated in both groups of subjects for the duration of the experiment as well as through the postbedrest period.

CONCLUDING REMARKS

The data indicate that altering the hydrostatic pressure for long periods of time causes a reduction in the circulating hydrocortisone rhythm amplitude, increases in thyroid hormone levels (despite the expected reduction in metabolic rate), and a desynchronization of thyroid rhythms with light as well as steroids. The diurnal rhythm in plasma steroids has been described as being associated with sleep rather than with the light schedule. The dissociation of the thyroid rhythms from both the hydrocortisone rhythm and the light-and-activity schedule, and the prompt reassociation of the two rhythms in the postbedrest ambulatory period suggest that the thyroid rhythm may be posture dependent.

Exercise has been used or at least suggested as the prophylactic or corrective measure for cardiovascular and metabolic changes occurring during bedrest and weightlessness. This corrective effect was certainly not evident in this study in which neither a vigorous exercise program nor the 10-day postbedrest ambulatory period prevented or corrected the heart rate, temperature, and endocrine changes induced by 56 days of absolute bedrest. It would appear from these data that corrective measures other than exercise should be sought.

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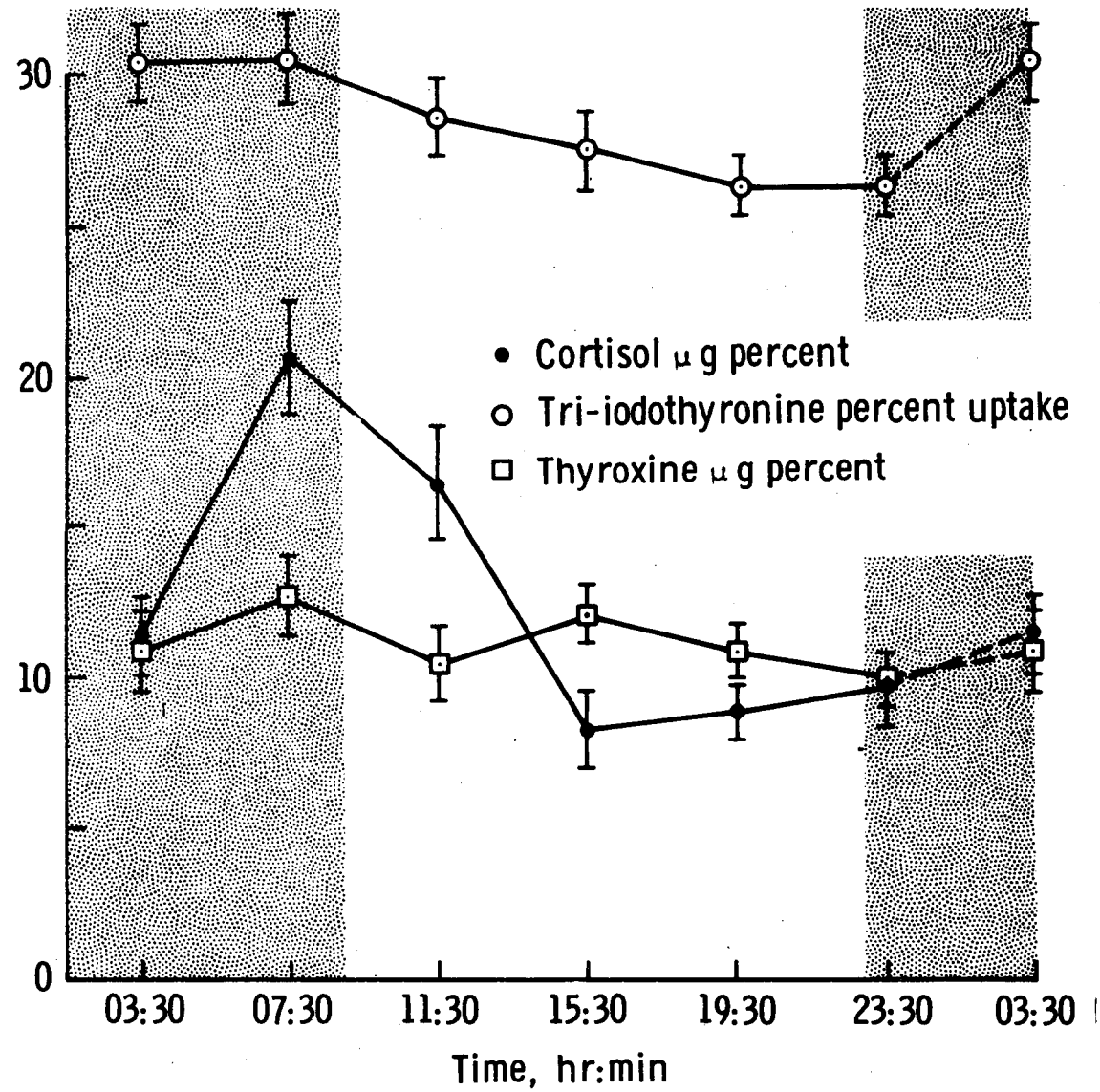

Figure 5-1.- Diurnal rhythms in mean circulating cortisol, thyroxine, and tri-iodothyronine in eight normal ambulatory subjects. Vertical lines represent standard error of the mean; stippled areas represent lights-off periods.

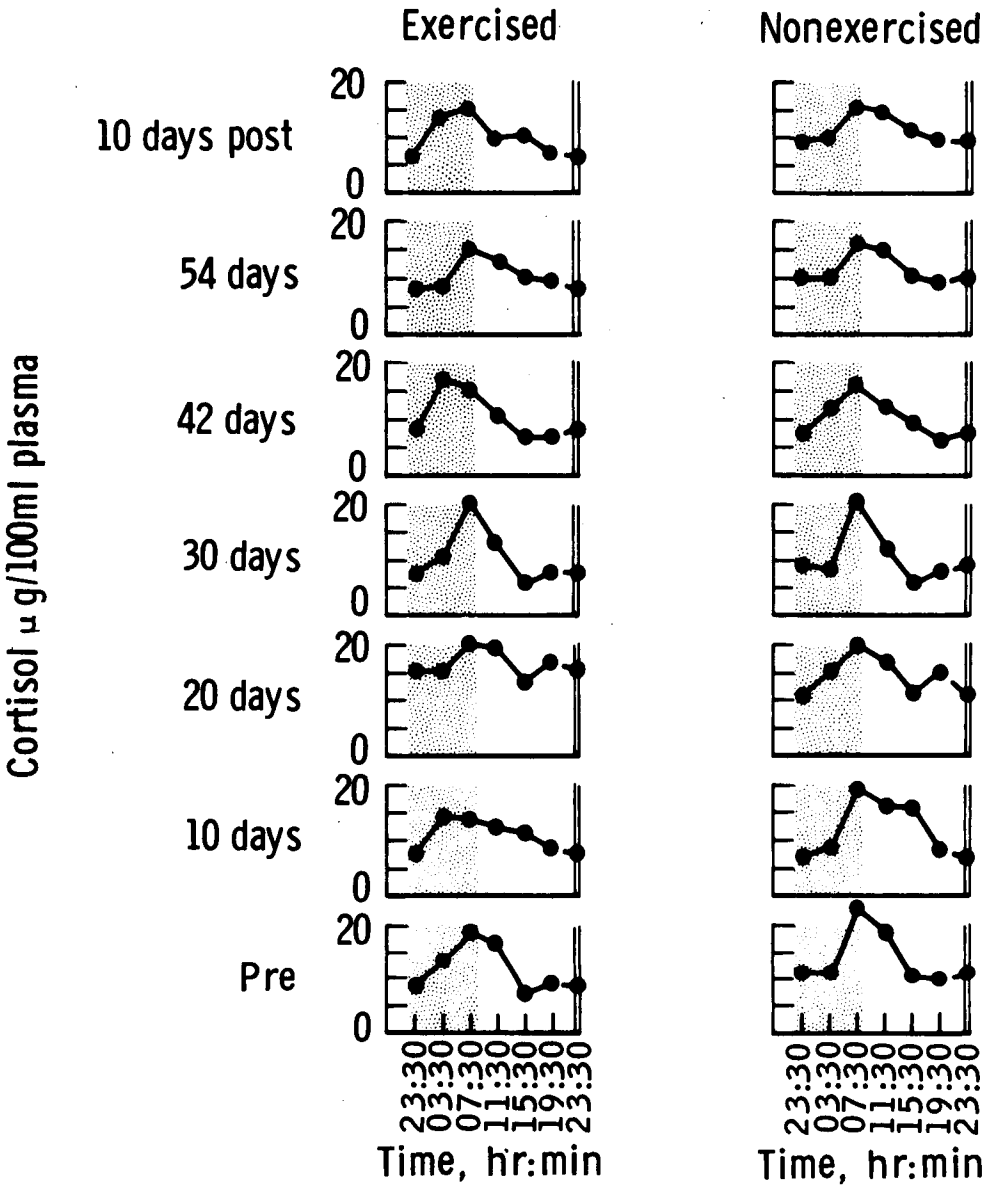

Figure 5-2.- Plasma cortisol rhythm in four exercised and four nonexercised subjects before, during, and after 56 days of bedrest. Stippled areas represent lights-off periods.

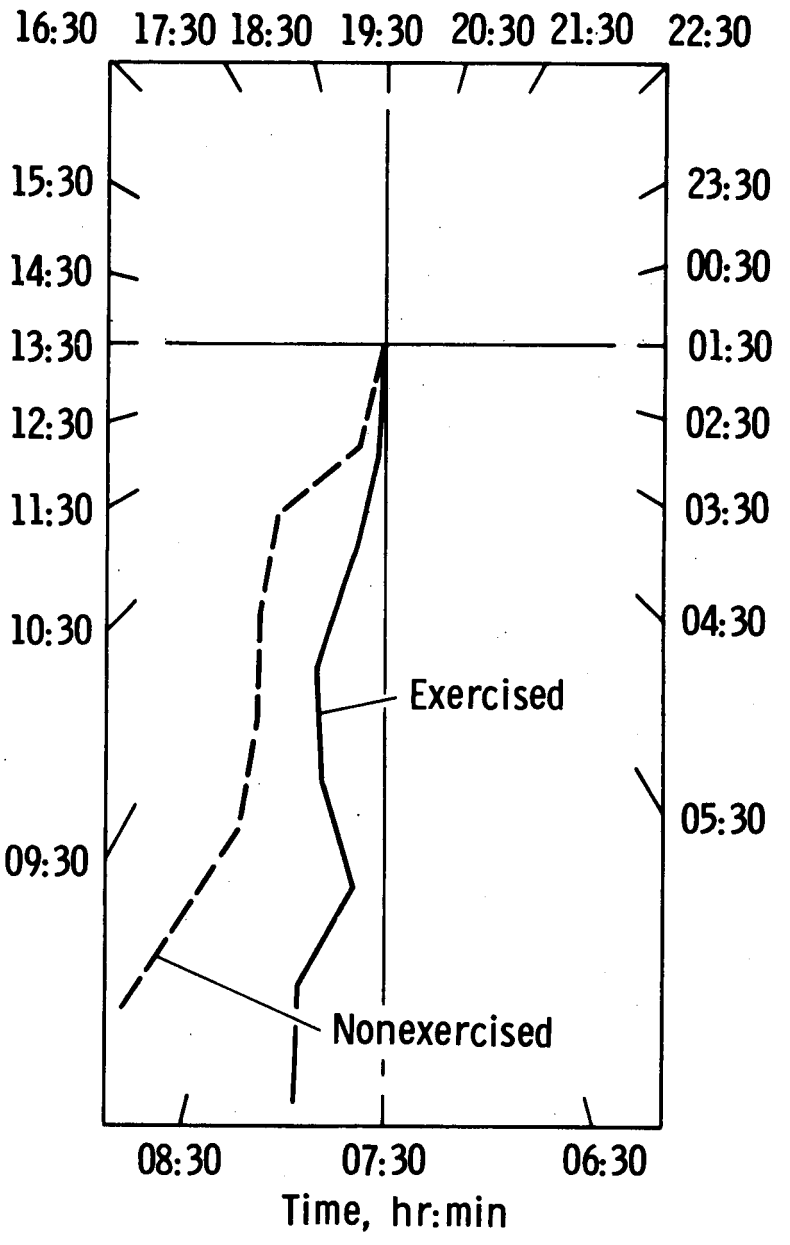

Figure 5-3.- Summation dial of plasma cortisol rhythm in four exercised and four nonexercised subjects during 56 days of bedrest.

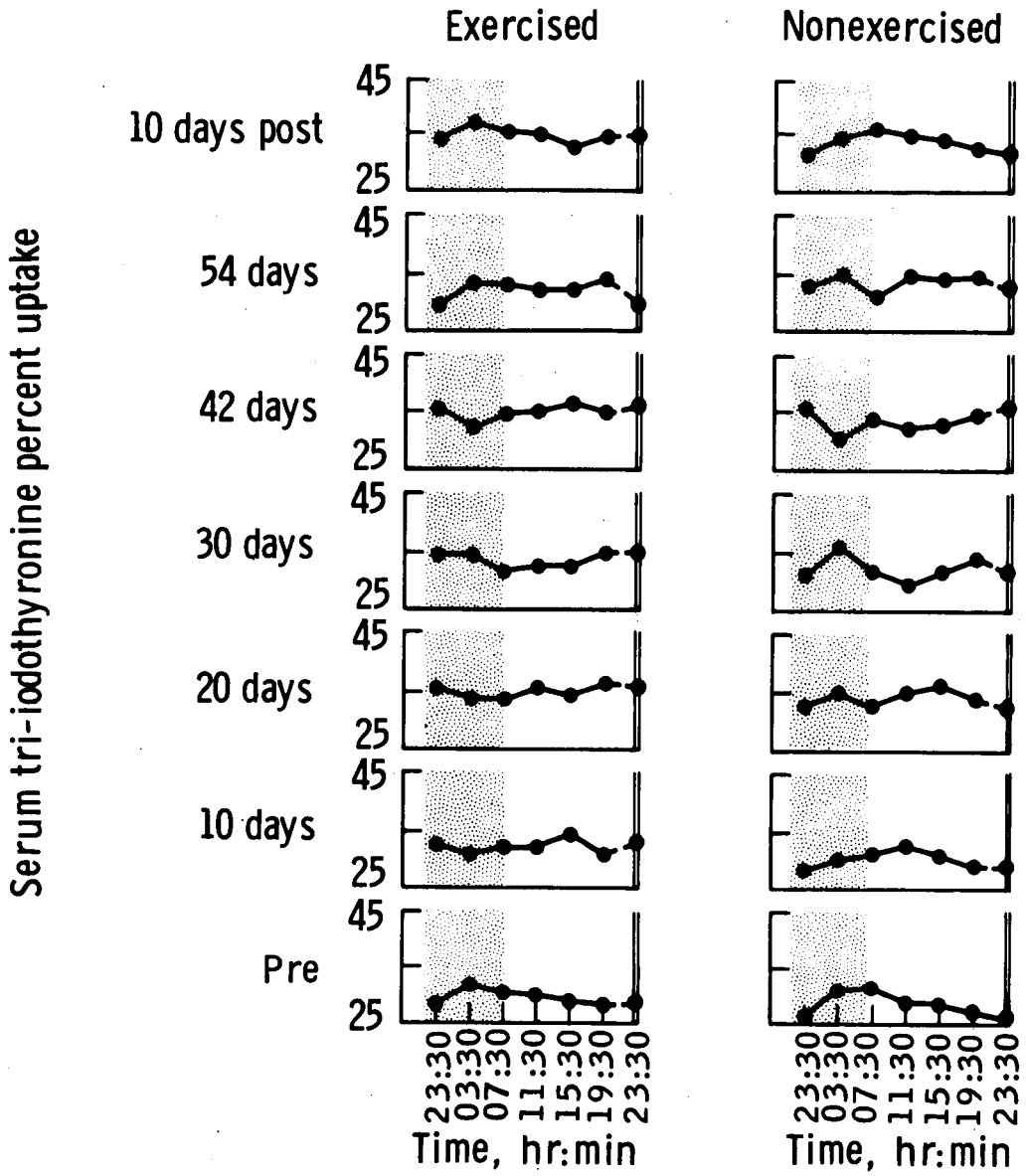

Figure 5-4.- Serum tri-iodothyronine in four exercised and four nonexercised subjects before, during, and after 56 days of bedrest. Stippled areas represent lights-off periods.

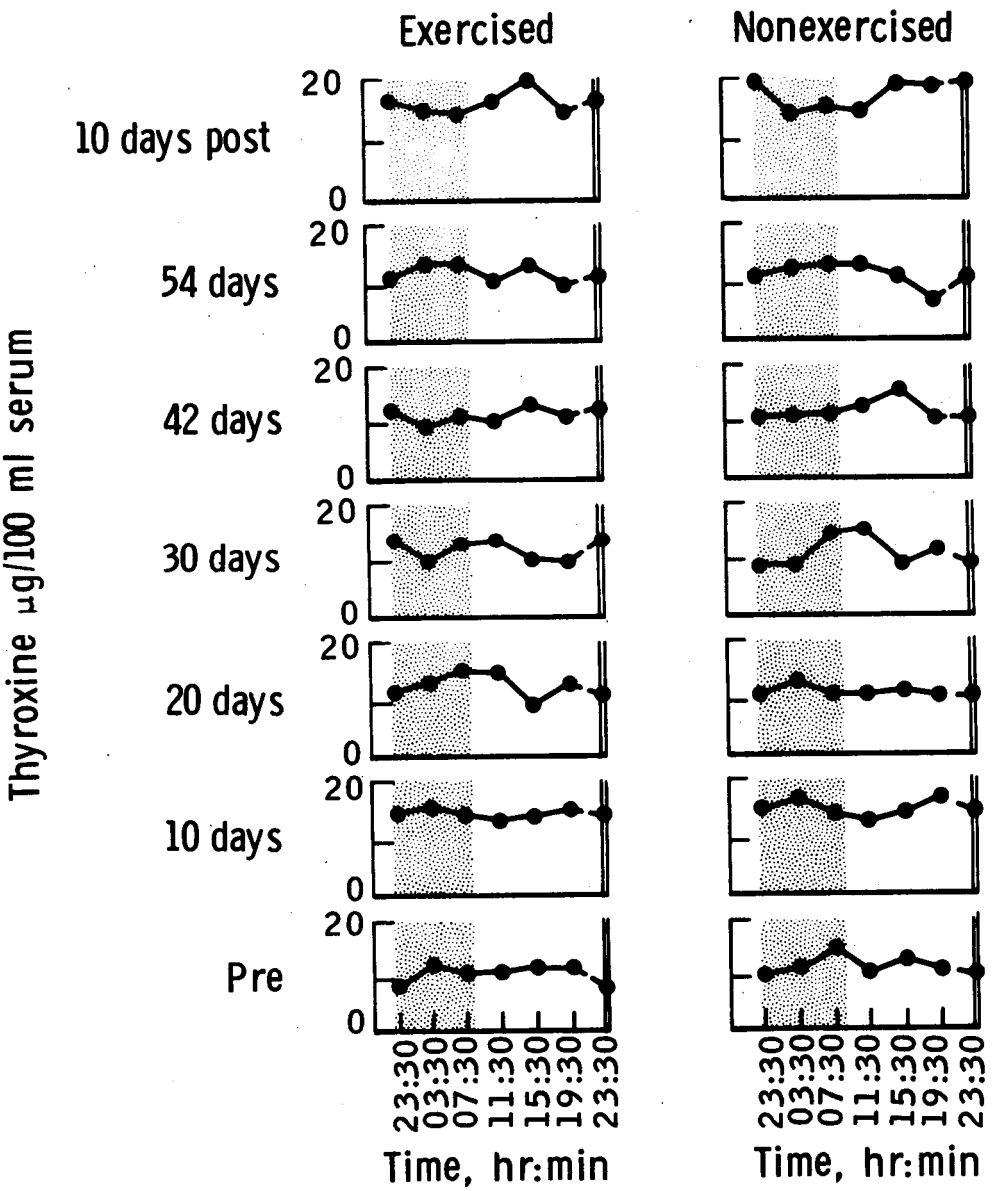

Figure 5-5.- Serum thyroxine in four exercised and four non-exercised subjects before, during, and after 56 days of bedrest. Stippled areas represent lights-off periods.

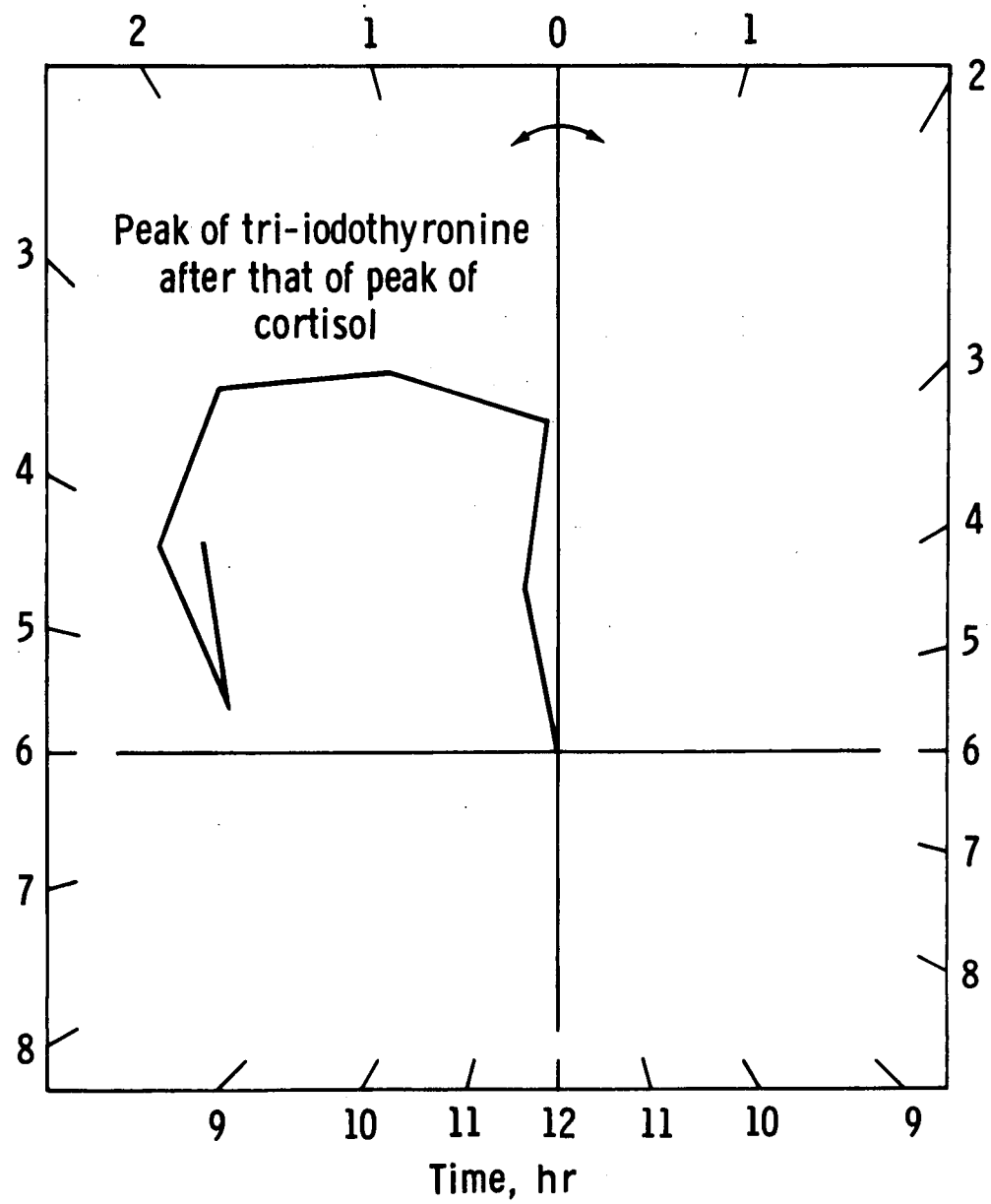

Figure 5-6.- Difference-vector summation dial correlating control and tri-iodothyronine rhythms in the nonexercised subjects at various stages during bedrest.

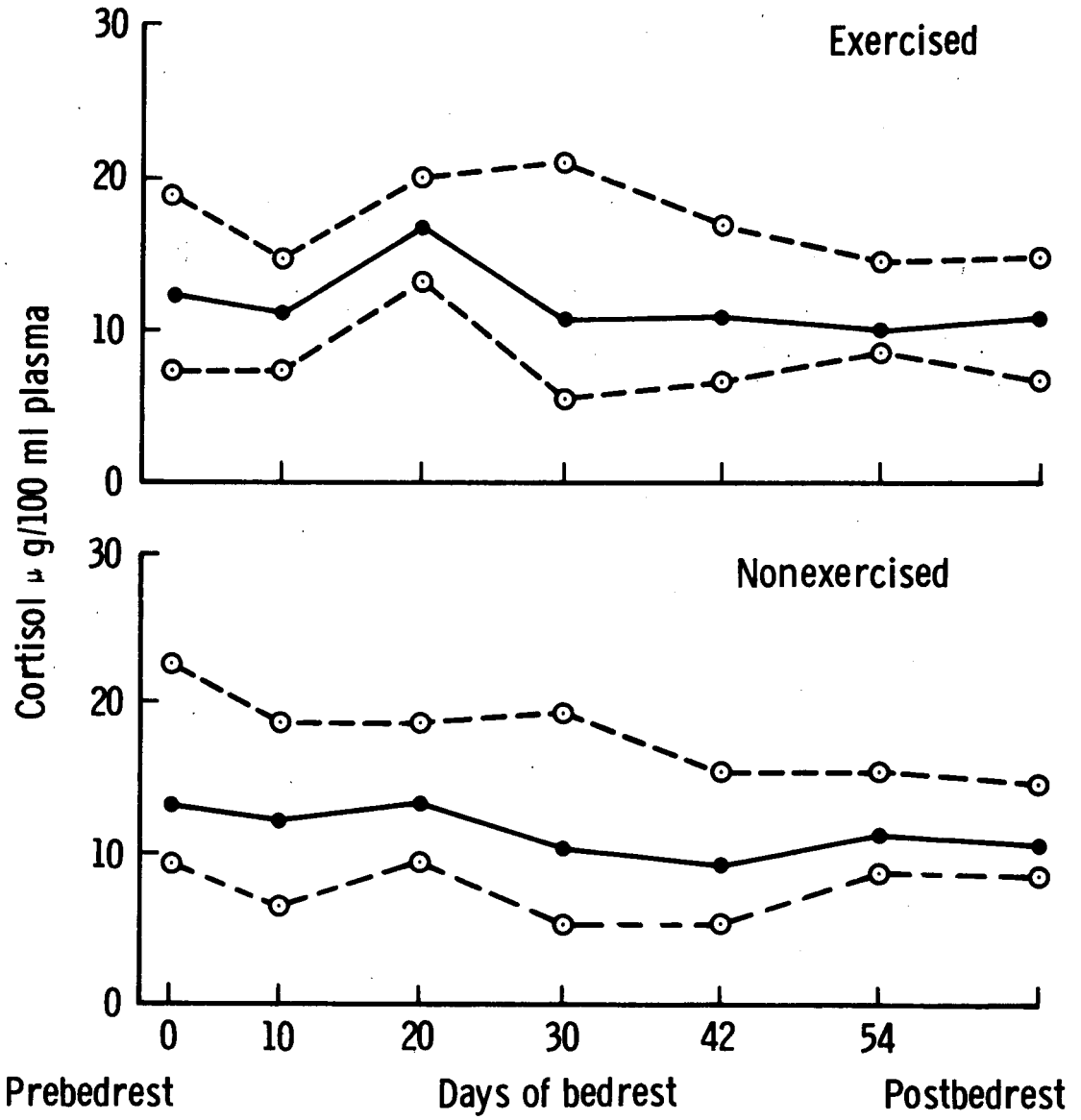

Figure 5-7.- Mean circulating cortisol (solid line) per 48-hour sampling period in four exercised and four nonexercised subjects. Open circles and dashed lines represent the mean maximal and minimal concentrations for that period irrespective of the hour of the day that they occurred.

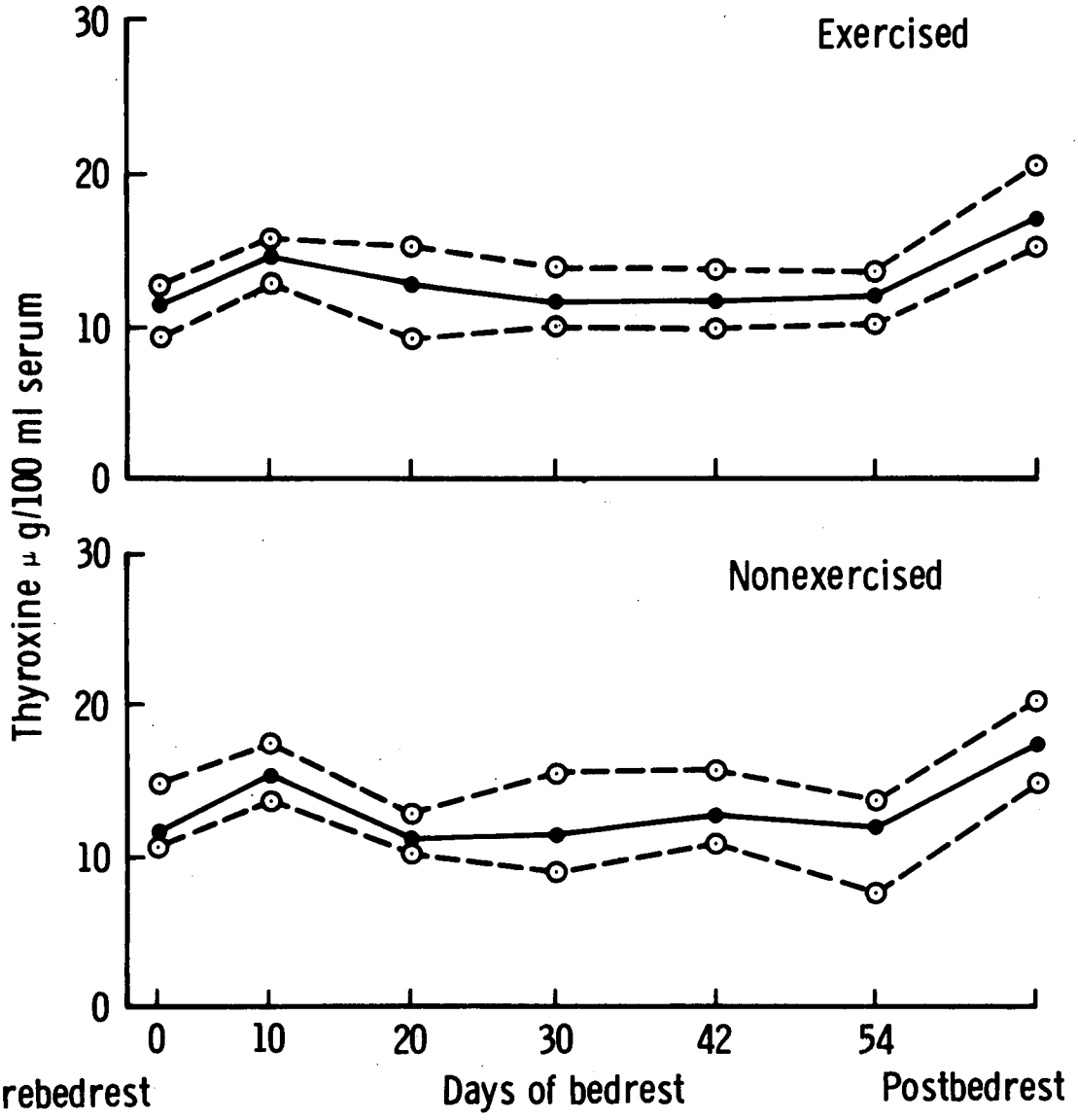

Figure 5-8.- Mean circulating thyroxine (solid line) per 48-hour sampling period in four exercised and four nonexercised subjects. Open circles and dashed lines represent the mean maximal and minimal concentrations for that period irrespective of the hour of the day that they occurred.

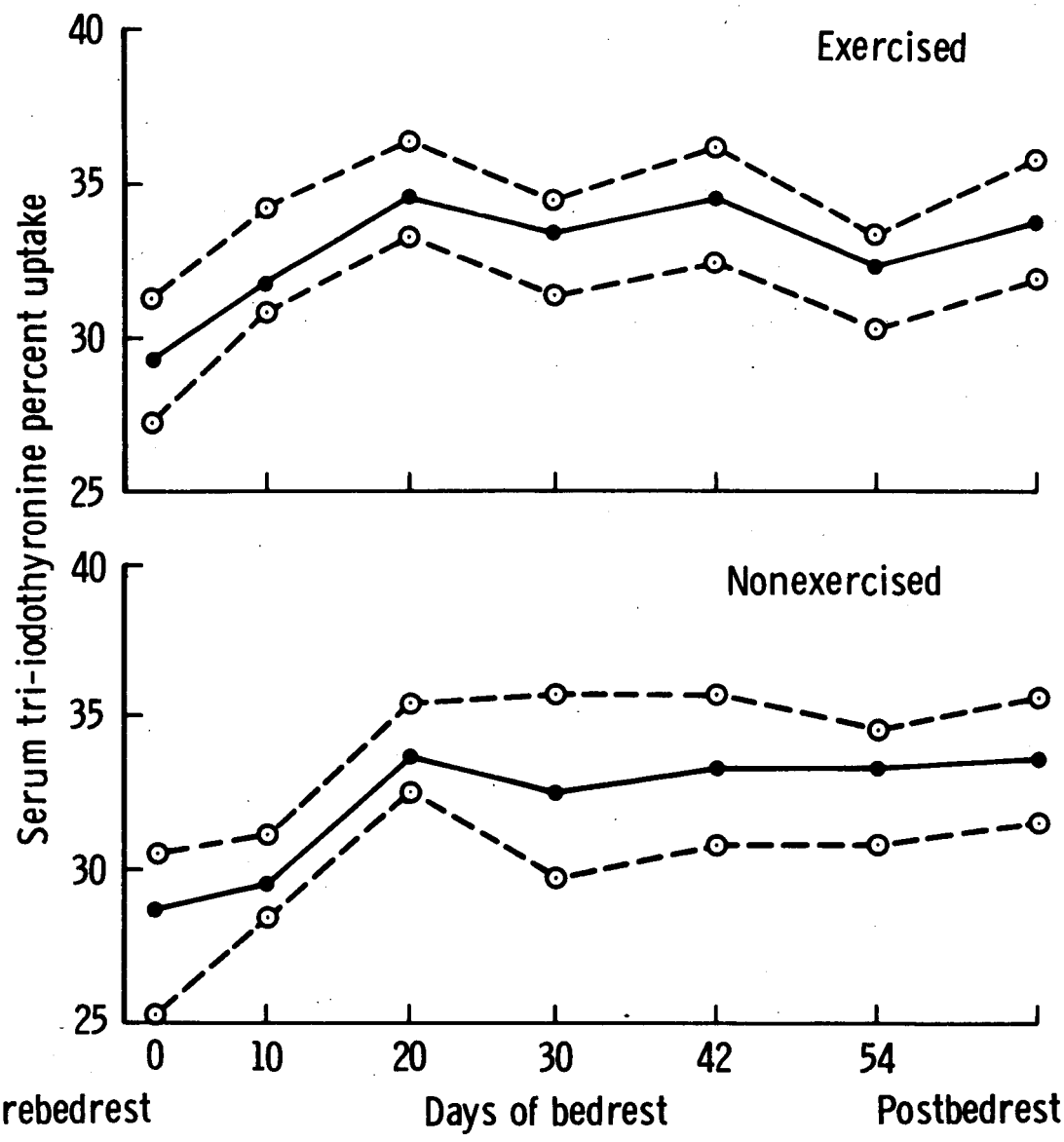

Figure 5-9.- Mean circulating tri-iodothyronine (solid line) per 48-hour sampling period in four exercised and four nonexercised subjects. Open circles and dashed lines represent the mean maximal and minimal concentrations for that period irrespective of the hour of the day that they occurred.

6. RADIOIMMUNOASSAY OF ANTIDIURETIC HORMONE

by Myron Miller, M.D.*

Many problems in the adaption of man to space appear to be related to changes in plasma volume and in total-body-water regulation, with many other changes taking place secondarily. The work on the development of a radioimmunoassay system for urinary antidiuretic hormone (ADH) will be discussed in this section. (The application of this system to the measurement of urinary ADH in the study of man in space is presented in section 3.) The data to be shown will highlight some of the features of the assay and some of the evidence that demonstrates that changes in urinary ADH excretion occurs after physiological stimulation and that these changes can be measured.

The assay system uses an antibody that was developed by immunizing rabbits with lysine vasopressin (LVP), which is one amino acid different from arginine vasopressin (AVP), the natural ADH of man. The LVP was coupled to bovine serum albumin using the carbodiimide condensation reaction. The antibody obtained reacted not only with the original antigen, LVP, but also cross-reacted with AVP. Assays were performed using ^{125}I-labeled LVP as the antibody and synthetic AVP as the standard. Separation of antibody bound from free LVP-^{125}I was originally performed using the double-antibody-precipitation procedure (fig. 6-1). The lower limit of sensitivity is approximately 25 μU AVP/ml and a dose-response curve occurs from 25 to 10 000 μU AVP/ml. The lower limit of sensitivity is equivalent to 62 pg AVP/ml. Greater sensitivity was noted when LVP was used in place of AVP as the standard.

Presently, albumin-coated charcoal is used to separate antibody bound from free LVP-^{125}I (fig. 6-2). This charcoal method produces a dose-response curve very similar to the curve obtained with the double-antibody procedure. The charcoal method has many advantages in that it allows the assays to be performed over a shorter period of time, at lesser cost, and with decreased variability. On some occasions and with some antisera, sensitivity to a level of 10 μU/ml is achieved occasionally.

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The antibody produced is capable of cross-reacting with a variety of neurohypophyseal peptides (fig. 6-3). The LVP, the material used to immunize the animals, exhibits the greatest sensitivity with approximately a twofold increase in sensitivity over AVP. Phenylalanine-2 LVP, which differs from the LVP only in the substitution of a phyenylalanine in position 2, also cross-reacts in the system. Oxytocin has no cross-reactivity in this assay.

The next series of studies concerns some of the biological work performed to validate the methodology. Posterior pituitary glands were obtained from rats that had been subjected to varying degrees of dehydration so that the content of ADH in the posterior lobe varied considerably. These pituitaries were assayed simultaneously using the immunoassay and a standard pressor-bioassay procedure (fig. 6-4). The regression line, obtained by plotting values determined by immunoassay against those determined by bioassay, illustrates that excellent agreement exists between the two assay methods. In general, the immunoassay results in values that are slightly higher than those of the bioassay levels.

A pool of plasma from normal human subjects was obtained and graded amounts of AVP were added to aliquots of the plasma. The plasmas were then extracted and assayed using both the immunoassay system and the antidiuretic bioassay system (fig. 6-5). In these assays, which are in the microunit range, good agreement again exists between the values obtained using the immunoassay and the bioassay procedures. The immunoassay values are slightly higher than the bioassay values. Plasma samples, to which no ADH had been added, contained approximately 5 μU AVP/ml of plasma (as determined by bioassay), and the same samples contained from 5 to 25 μU AVP/ml of plasma (as determined by immunoassay). This disparity in values indicates the detection by the immunoassay of some component that was not detectable in the bioassay system. This component may represent the presence of partially degraded forms of vasopressin that are no longer biologically active but that retain immunoreactivity. Other data indicate that the biological activity of vasopressin can be altered while the immunological activity is still retained. The disulfide link in vasopressin can be broken without affecting the immunological activity of the vasopressin, whereas such a break will destroy most of the biological activity of ADH. Other changes that will decrease the biological activity can be made in the molecule, but the changes have a lesser effect on the immunological activity. Thus, it is not difficult to obtain disassociation between the two activities.

Urine specimens were obtained from rats that were normal or that had varying degrees of diabetes insipidus (fig. 6-6). After extraction of the ADH, the samples were assayed simultaneously using the immunoassay and the antidiuretic bioassay. Again, excellent agreement occurred between the two assay procedures. The immunoassay revealed a small amount of activity that was not detected by the bioassay.

The lower limit of sensitivity of the immunoassay is approximately 25 μU AVP/ml. Therefore, extraction and concentration of these fluids were required to obtain a reliable measurement of the amount of ADH in biological fluids. Urine was acidified with acetic acid to a pH of 4.5 and then passed through a column of the ion-exchange-resin CG-50. The ADH is adsorbed by the resin while the urine passes through. The columns were then washed once with water and again with 50 percent alcohol (both of these solutions were at a pH of 4.5). The wash results in the removal of salts and other small peptides, and ADH remains on the column. When 75 percent alcohol, at a pH of 2, is passed through the column, ADH is quantitatively removed and the eluate is then evaporated and stored for later assay.

The percent recovery achieved using this procedure is listed in table 6-I. When LVP-^{125}I was used and the radioactivity recovered at the end of the extractive procedure was determined, 89.4 percent of the LVP-^{125}I applied to the column was recovered. When known amounts of AVP were added to urine obtained from patients with complete diabetes insipidus and the urine was passed through the columns and then assayed, the mean recovery rate was 98.5 percent. The time required for the extractive procedure is several hours. Consequently, a specimen can be collected, processed, evaporated, and stored in a single day.

Biological experiments were performed using the urinary extraction-and-concentration procedure and the immunoassay method of measuring ADH. Urinary ADH excretion was determined in Brattleboro rats that were either normal, had complete diabetes insipidus, or had a heterozygous form of diabetes insipidus in which approximately one-half the normal amount of vasopressin is present in the posterior pituitary gland. The relationship observed between the posterior pituitary ADH content and urinary ADH excretion was excellent. In normal animals, mean urinary ADH excretion was 4.6 mU AVP/day whereas, in the animals with complete diabetes insipidus, only small amounts of immunoreactivity — equivalent to approximately 100 μU of AVP/day (fig. 6-7) — were observed. The material in the animals with complete diabetes insipidus is probably not AVP because the material does not have biological activity. The animals with the heterozygous form of diabetes insipidus (which have approximately one-half the normal amount of ADH in the posterior pituitary) also excrete about one-half the normal amount of ADH in the urine — 2.3 μU/day. Therefore, an excellent correlation exists between urinary ADH excretion and neurohypophyseal ADH content.

Close correlation also exists between the urinary ADH excretion and urine osmolality, and between the urinary ADH excretion and urine volume (fig. 6-8). In the rats with diabetes insipidus (which have large volumes of very dilute urine) the rate of ADH excretion is very low. Normal rats

(which have a urine osmolality of approximately 2300 mOsm/kg) have a high rate of excretion of ADH and an inverse correlation with the urine volume, as would be expected.

In normal animals, in response to a 4-day period of dehydration, the urinary ADH excretion rises approximately fivefold from a base-line value of 3.6 to 18.2 mU/day (fig. 6-9). When these rats are subsequently given access to water, the urinary ADH values promptly fall back to a level that is even lower than in the basal state. The animals evidently drink excessively in response to the previous dehydration and, consequently, the ADH-release mechanism is effectively inhibited. In the animals with the heterozygous form of diabetes insipidus, the same phenomenon occurs but to a lesser degree. These animals excrete approximately 2 mU AVP/day and undergo a threefold increase to 5.6 mU/day in response to dehydration. After the rats have had access to water, the ADH excretion falls sharply to less than the basal values. The animals with complete diabetes insipidus do not increase the excretion of ADH-like material in response to dehydration and, when the animals again are given access to water, the immunoreactive material in the urine falls significantly, suggesting that the material being measured in these animals originate in the neurohypophyseal system. This reaction may represent a precursor or abnormal peptide that does have some immunoreactivity in this system.

Some preliminary data have been obtained in humans (fig. 6-10). In a group of 25 normal subjects, urine specimens were collected every 24 hours from individuals while under conditions of normal activity, eating, and drinking. The mean osmolality of these urine specimens was 940 mOsm/kg. The mean ADH excretion was 1.3 mU/hr over the 24-hour period. In individuals who were given water loads so that their urine osmolality fell to a mean of 67 mOsm/kg, ADH disappeared entirely from the urine. Two patients with inappropriate ADH syndrome secondary to carcinoma of the lung were also studied. One patient had a markedly elevated ADH excretion, far above that observed in normal individuals. The second patient had a value in the same range as normal subjects. However, the plasma osmolality in the second patient was approximately 252 mOsm/kg — a value far below the normal plasma osmolality of 285 to 288 mOsm/kg. Thus, the urine ADH value obtained was inappropriately high. In three patients with diabetes insipidus who had a mean urine osmolality of 90 mOsm/kg, no detectable ADH was found in the urine.

The response of a normal individual to an oral water load is illustrated in figure 6-11. After a period of 18 hours of dehydration, urinary ADH excretion was 2.8 mU/hr. After administration of the water load, a progressive decrease in urine osmolality from 950 mOsm/kg to 70 mOsm/kg was observed. Concurrently, diuresis began and ADH decreased, so that, at the end of an hour, ADH had disappeared from the urine. The water load was maintained for a period during which the urine osmolality remained low and ADH was absent. As the water load was allowed to be

excreted and as the urine osmolality began to rise again, urine volume fell, and ADH reappeared in the urine. The ADH reappeared when urine osmolality was still below the level of the plasma osmolality. Thus, ADH release appeared to begin to occur when the level of urine osmolality reached approximately 125 mOsm/kg.

The same type of study was performed in a patient with the inappropriate ADH syndrome resulting from an oat cell carcinoma of the lung (fig. 6-12). The patient, who had previously been water restricted, was given an acute water load at a time when his serum sodium was 130 mEq/liter. Essentially no change occurred in the urine osmolality. The urinary ADH decreased (but did not disappear) and remained present even though the serum sodium fell from 130 to 120 mEq/liter during this time. Thus, some degree of inhibition of the ADH excretion was observed in response to the water load, suggesting that the tumor in this patient was partially responsive to a normal regulatory mechanism.

The methodology for determination of urinary ADH was evidently verified by the studies performed. Urine can be concentrated to raise the level of ADH in the specimen to the level of sensitivity of the assay. By using this technique, it is possible to measure quantitatively changes in urinary ADH excretion that would be expected to occur in response to physiological stimuli and thus make possible study of the role of ADH in water regulation.

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TABLE 6-I.- RECOVERY OF VASOPRESSIN AFTER EXTRACTION ON CT-50 COLUMN

Assay	LVP-^{125}I recovered, percent	AVP recovered, percent
1	91.0	118
2	90.0	104
3	90.1	94
4	89.4	102
5	89.9	88
6	85.2	92
7	94.3	92
8	87.8	98
9	84.7	--
10	87.6	--
11	93.4	--
Mean	89.4	98.5
Standard deviation	3.0	9.5
Standard error of mean	0.9	3.4

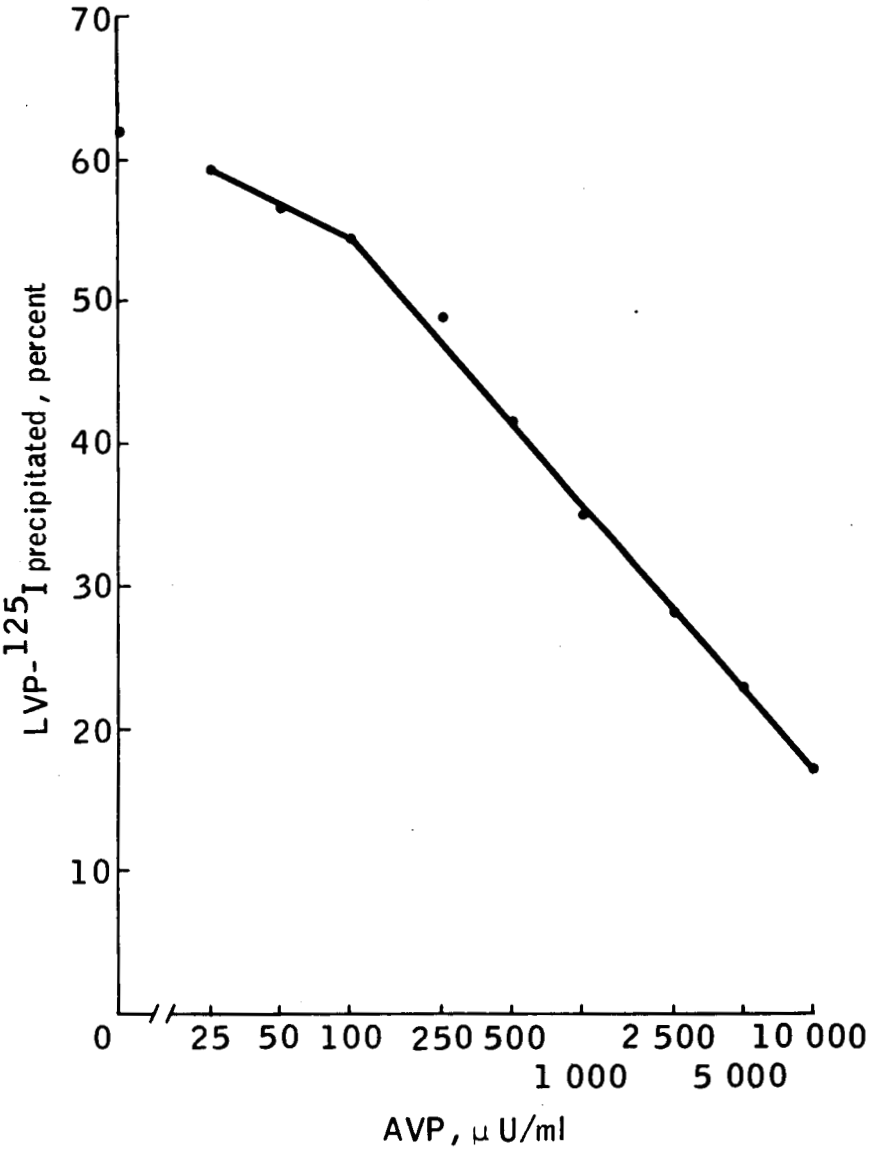

Figure 6-1.- Radioimmunoassay using AVP as the standard (semilogarithmic plot). The percent of LVP-^{125}I precipitated decreases as increasing amounts of AVP are added. The percent precipitated when no AVP is added is indicated by the point to the right of the plot.

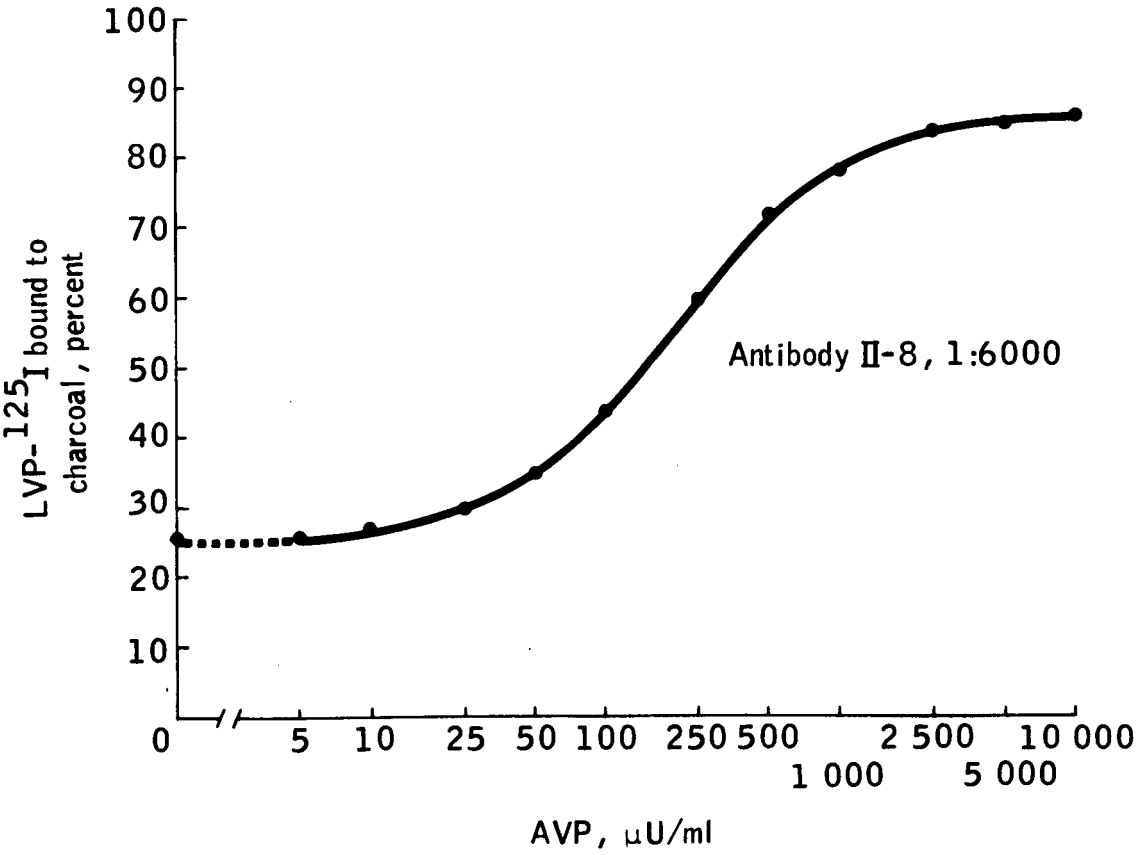

Figure 6-2.- Standard curve using albumin-coated charcoal to separate antibody bound from free LVP-^{125}I. The percent of LVP-^{125}I bound to charcoal increases as increasing amounts of AVP are added.

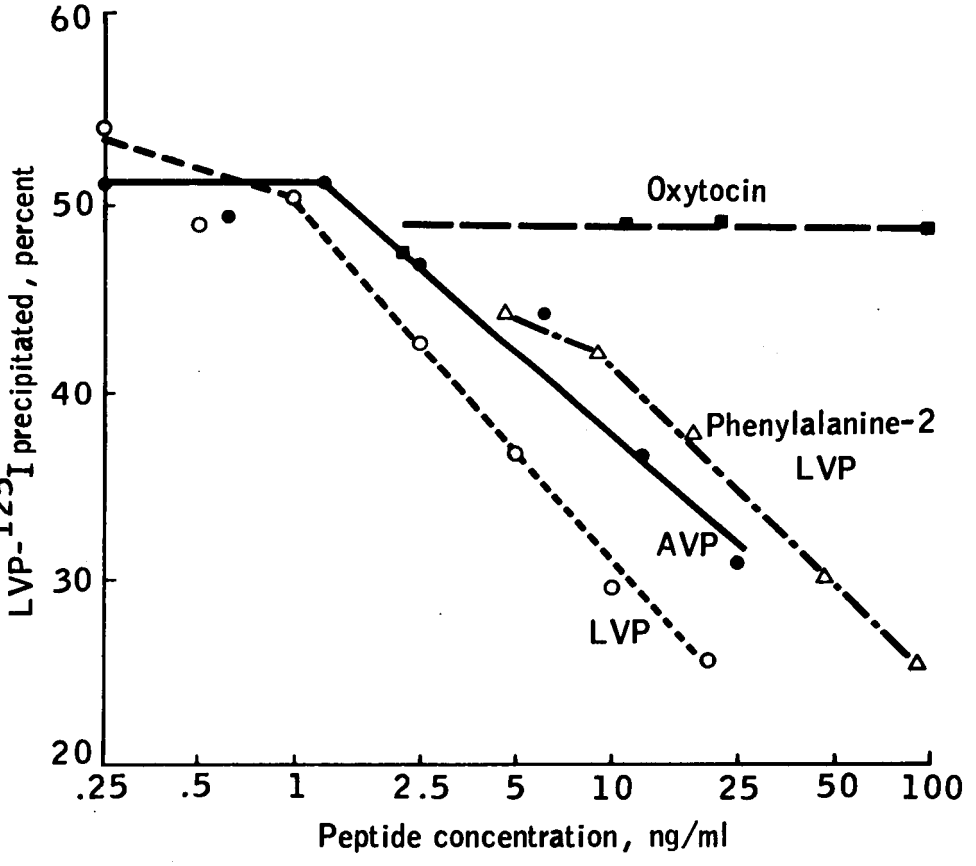

Figure 6-3.- Comparison of the relative ability of AVP, LVP, phenylalanine-2 LVP, and oxytocin to decrease the percent of LVP-^{125}I precipitated.

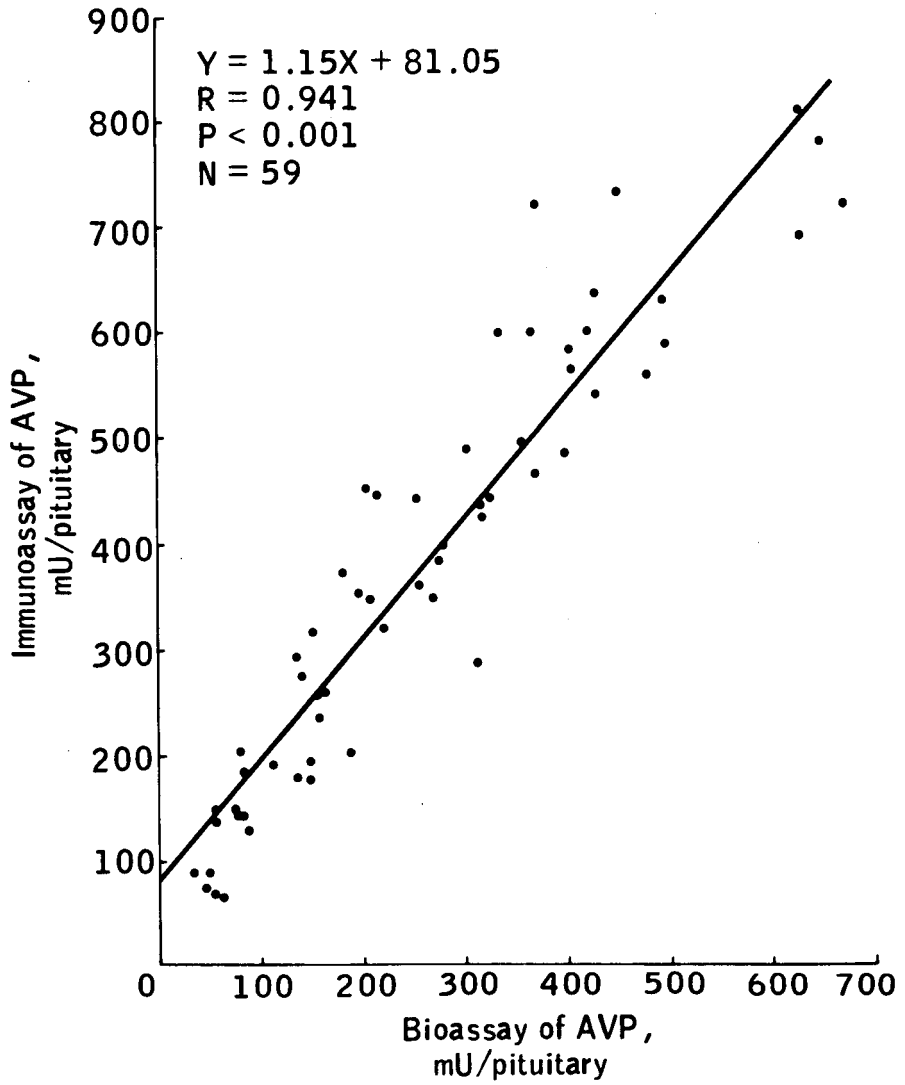

Figure 6-4.- Comparison of rat posterior-pituitary content of AVP as determined by immunoassay with AVP content of the same specimen as determined by bioassay. Pituitaries were obtained from normally hydrated rats and from animals subjected to water deprivation for as long as 4 days.

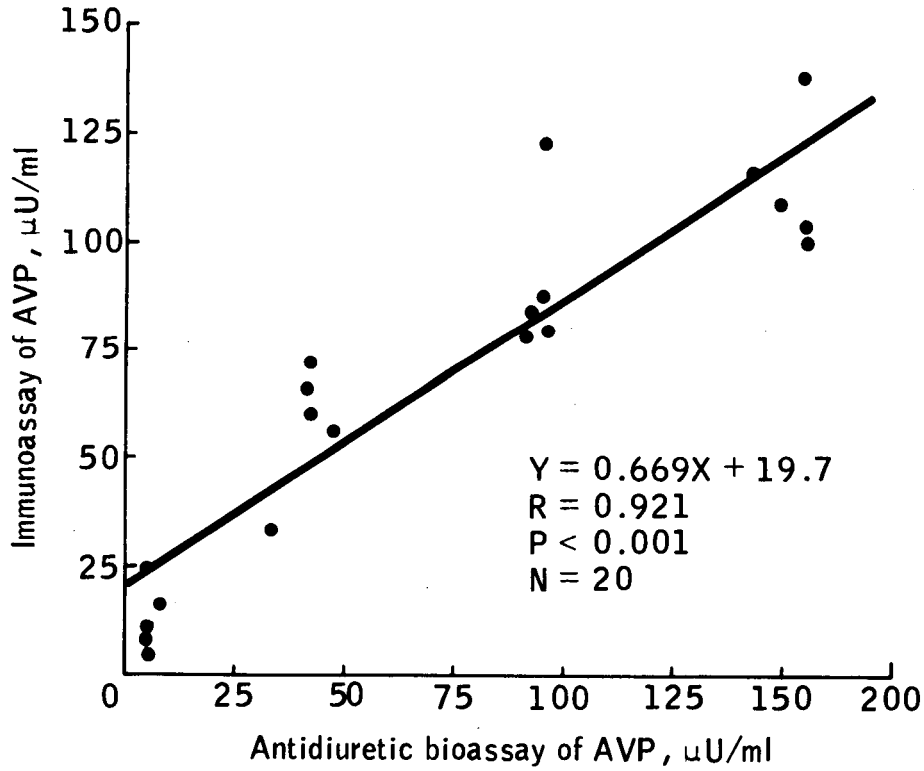

Figure 6-5.- Comparison of plasma content of AVP as determined by immunoassay and by antidiuretic bioassay of the same specimens. Known amounts of AVP were added to aliquots of a pooled plasma sample.

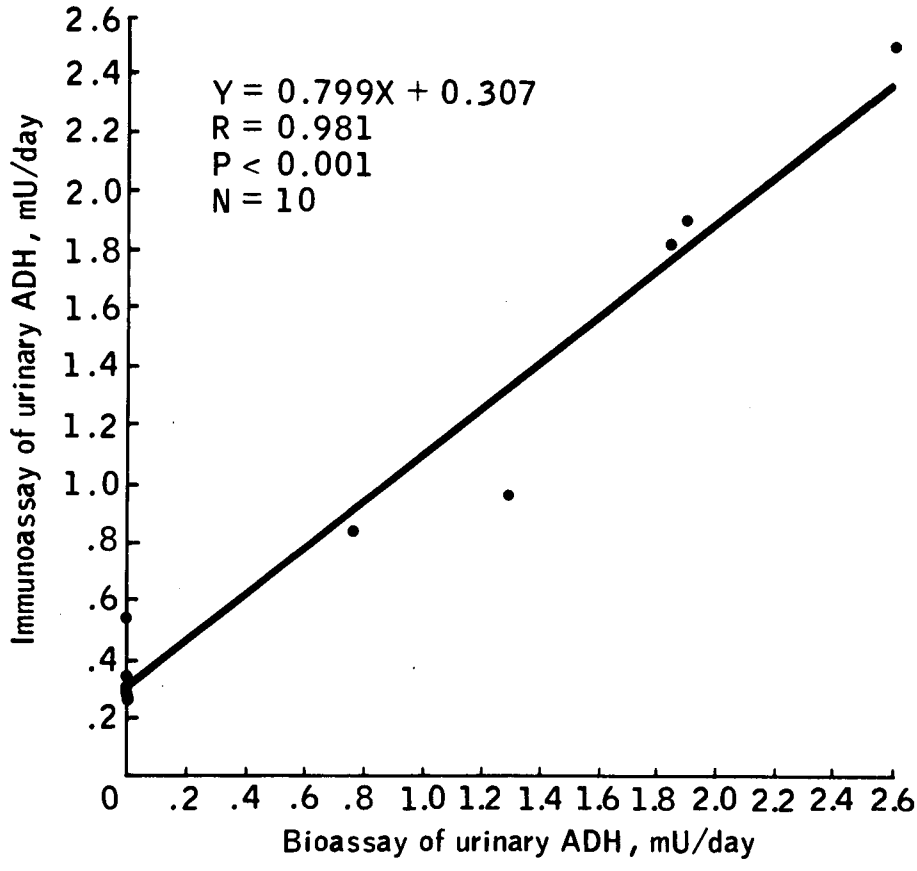

Figure 6-6.- Comparison of urinary ADH content as determined by immunoassay and by antidiuretic bioassay of the same specimens. Urine was obtained from rats that were normal or had varying degrees of diabetes insipidus.

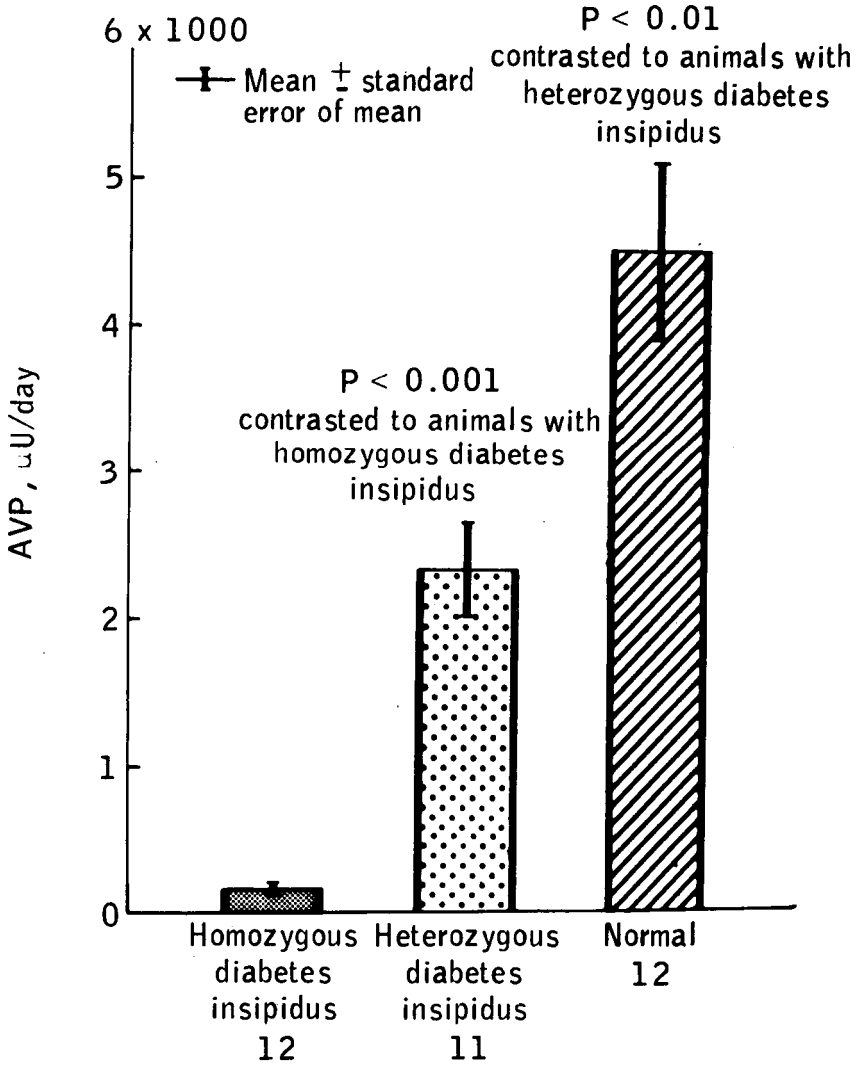

Figure 6-7.- The 24-hour urinary excretion of AVP in normal rats and in rats heterozygous or homozygous for diabetes insipidus.

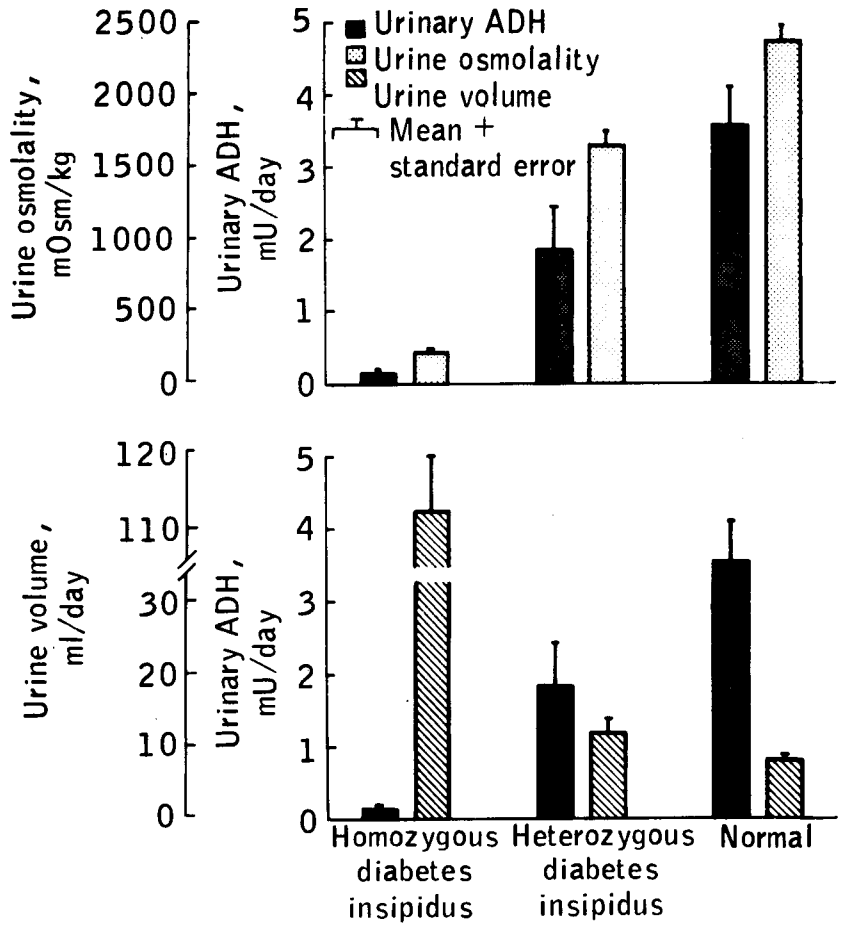

Figure 6-8.- Relationship between 24-hour urinary ADH excretion and urine osmolality (top) and 24-hour urine volume (bottom) in normal rats and rats heterozygous or homozygous for diabetes insipidus.

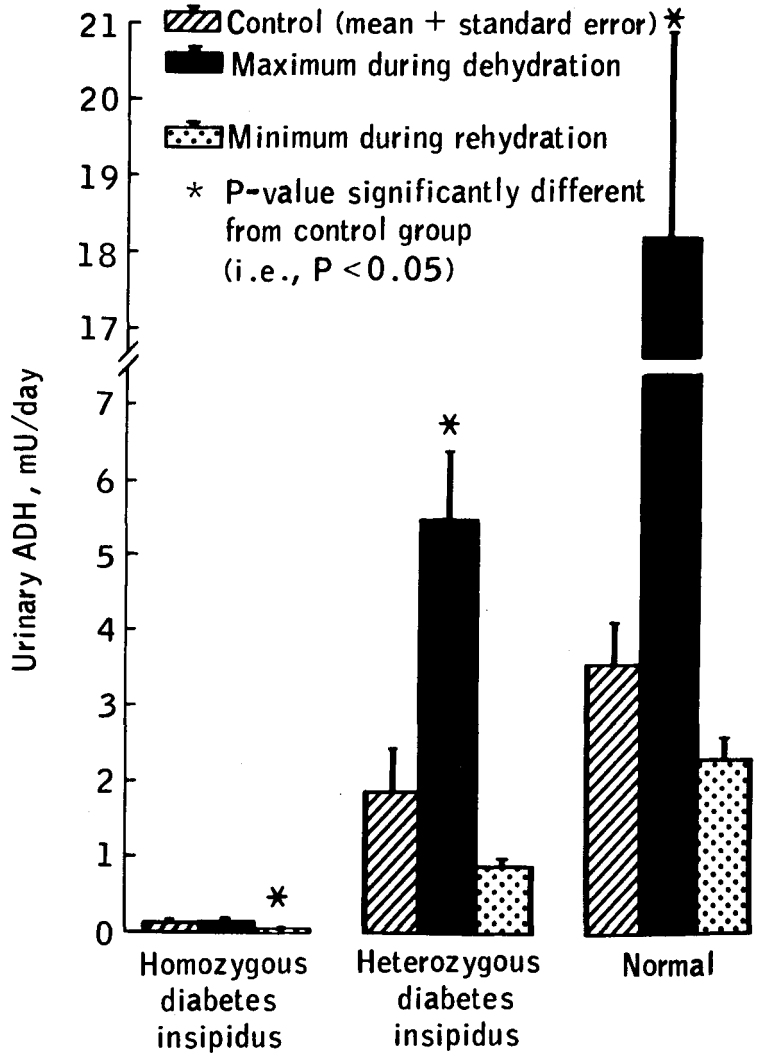

Figure 6-9.- Urinary ADH excretion response for 24-hour period in the three types of rats to water deprivation and to subsequent free access to water. Normal and heterozygous rats were dehydrated for 4 days while rats homozygous for diabetes insipidus were dehydrated for 2 days.

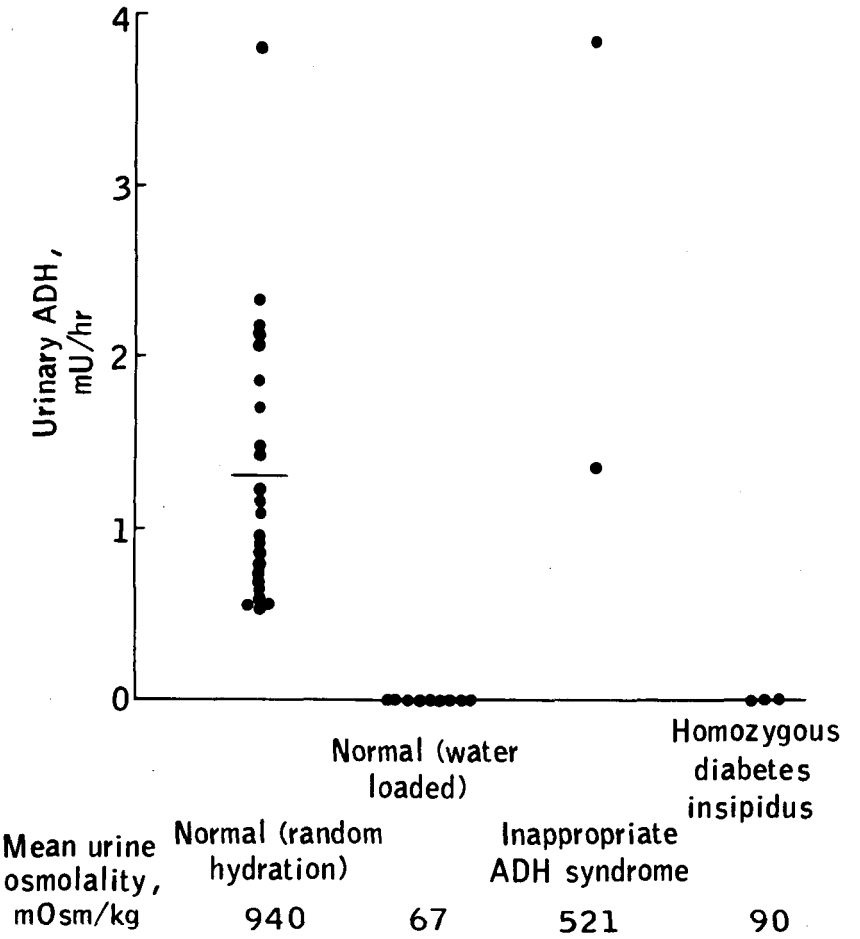

Figure 6-10.- Urinary ADH excretion in human subjects. The normal individuals were studied under conditions of normal activity, eating, and drinking; and again after oral water loading. Two patients with inappropriate ADH syndrome resulting from an oat cell carcinoma of the lung and three patients with severe central diabetes insipidus were also studied.

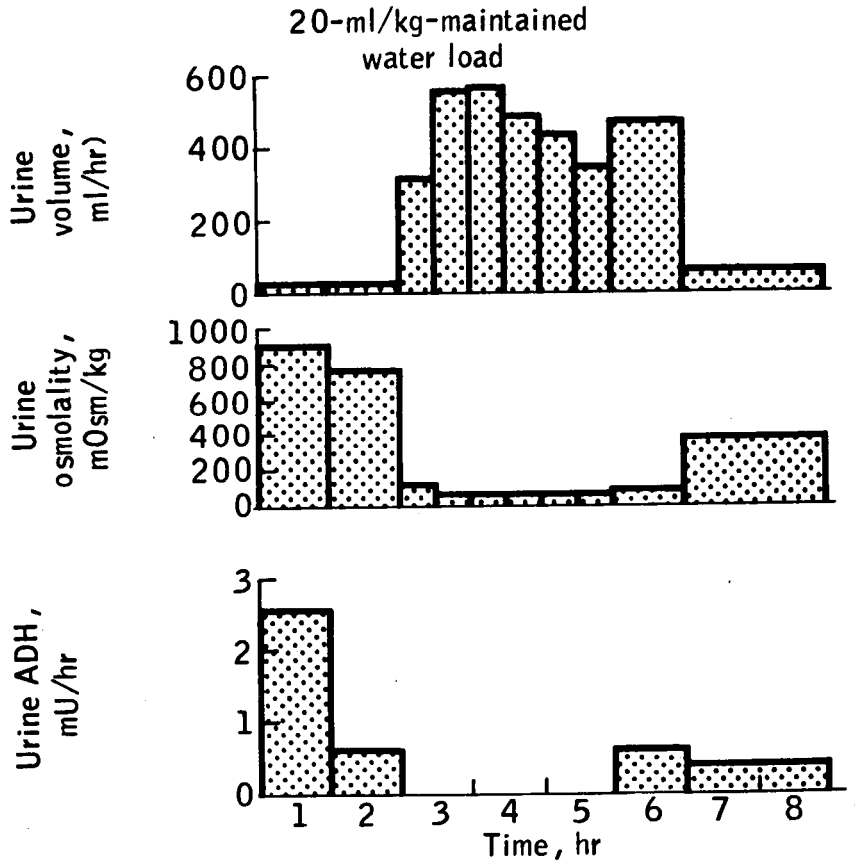

Figure 6-11.- Response of urine volume, osmolality, and ADH excretion to a maintained oral water load in a normal subject. Urinary ADH excretion becomes undetectable during the period of maximum diuresis and reappears following discontinuation of the water load.

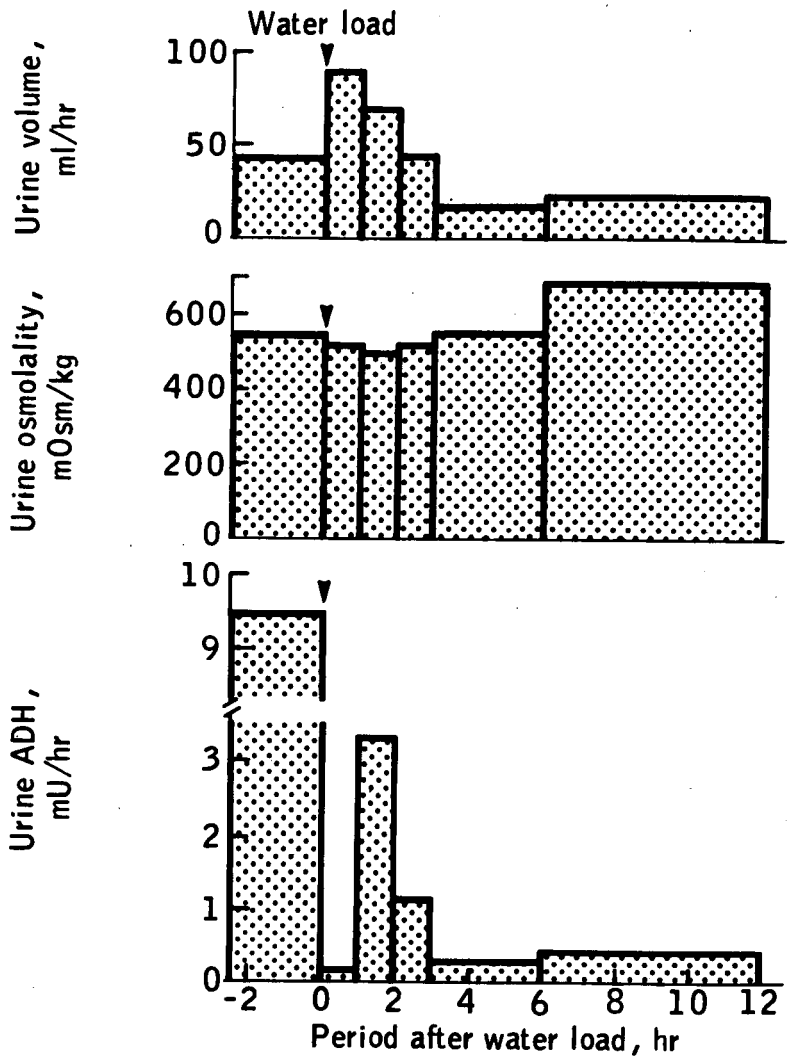

Figure 6-12.- Response of urine volume, osmolality, and ADH excretion to a single water load in a 65-year-old male patient with inappropriate ADH syndrome resulting from an oat cell carcinoma of the lung. Although urinary ADH excretion decreased, it still remained detectable despite marked hyponatremia and plasma hypoosmolality.

7. HUMAN VASOREGULATION BY RENIN, ANGIOTENSIN, AND ALDOSTERONE

By Edgar Haber, M.D.*

INTRODUCTION

A fundamental problem in the study of the hormonal control of vasoregulation is the capability to measure minute blood concentrations of circulating polypeptides and steroid hormones. The inability to quantify the levels of some hormones and the necessity to withdraw large volumes of blood to measure others have made detailed physiological investigations of the response of man to varying stress most difficult. It would be desirable to study man on a tilt table, under conditions of altered gravity, or under lower-body negative pressure, and to obtain multiple samples over brief intervals for use in the determination of aldosterone, angiotensin, and renin without changing blood volume appreciably. A general technique, radioimmunoassay, allows for such studies.

In the first part of this report, human physiological studies, using the radioimmunoassays for angiotensin I and II, will be discussed; in the second part, the development of a radioimmunoassay for aldosterone will be detailed. It is hoped that future studies combining these methods in human investigation will yield new insights into vasomotor regulation.

RENIN AND ANGIOTENSIN

Angiotensin II is measured by means of a direct radioimmunoassay that has the capability of quantifying normal levels (ref. 7-1). Renin activity is measured by generation of angiotensin I, the first product of the activity of this enzyme, during in vitro incubation of plasma, employing endogenous substrate (refs. 7-2 and 7-3). Renin activity is expressed as the amount of angiotensin I produced per hour of incubation.

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A study of 15 young, normal volunteers is summarized in figure 7-1. Blood samples were obtained on awakening, in the supine position, and after 4 hours of upright activity. Dietary intake of sodium was varied from severe restriction to excess. At the end of the study, a potent diuretic, furosimide, was given. Blood samples were obtained for the determination of angiotensin II and renin activity, and urine was collected for the determination of aldosterone. Urinary aldosterone was measured by use of a double-isotope-derivative method. These studies confirmed earlier observations (ref. 7-4) that renin activity responds to sodium intake and posture. Cohen et alii (ref. 7-4) demonstrated for the first time that circulating angiotensin II levels closely follow renin activity, although changes in renin activity appear to go through a wider range (factor of 10) than do venous angiotensin II levels. This difference may relate to blunting of venous angiotensin concentrations by extraction or degradation in capillary beds. Urinary aldosterone follows renin activity and angiotensin II changes in the expected manner because angiotensin II is believed to be the major hormone that regulates aldosterone level.

In summary, a change in "effective blood volume" by either upright posture or sodium restriction stimulates the release of renin by the kidney, which in turn results in angiotensin I production and conversion to angiotensin II and stimulation of the adrenal cortex by the angiotensin II to secrete aldosterone.

A vital question, previously unanswered, is whether this system affects moment-to-moment vasomotor regulation, or whether it affects only long-term "background" changes in blood volume. To answer this question, an experiment was designed in which normal human volunteers were subjected to short-term postural stress on a tilt table (ref. 7-5). Several patients with cardiac valvular disease also were studied; in these patients, renal-vein blood samples could be obtained during the course of ordinary cardiac catheterization. Pulse, blood pressure, and renin activity were measured after supine rest for 0.5 hour, followed by frequent samples after upright tilting to 80°.

Table 7-I shows that, although 27 of 36 subjects remained alert throughout 30 minutes of upright tilting, nine individuals fainted, necessitating termination of tilt earlier than 30 minutes. The individual responses of a typical normal responder and of a fainter are given in figures 7-2 and 7-3, respectively. A summary of the response of all volunteers is shown in figure 7-4. It is evident that the normal response was characterized by a rise in pulse rate, and in diastolic blood pressure, accompanied by a significant increase in renin activity. After return to the supine position, a rapid return of both hemodynamic parameters and renin activity to normal was observed. Conversely, the fainting group had a decrease in pulse rate, a narrowing of pulse pressure, a

reduction in systolic pressure, and a much less significant rise in renin activity. Renin activity rises paradoxically after return of the subject to the supine position. The noteworthy difference between normal responders and fainters with respect to maximum renin levels is documented in figure 7-5. Whereas normals increased by an average of 283 percent, the fainters achieved a maximal increase of only 49 percent, the rise generally occurring after return to the supine position. A rather normal response to tilting in an anephric subject, though no renin is present, is depicted in figure 7-6. Apparently, a normal postural response is possible in the absence of the renin mechanism.

Renal-vein measurements, shown in table 7-II, are indicative that renal-vein renins are well reflected in peripheral-vein renin levels, although the former are somewhat higher than the latter, as expected. The same kind of differences is evident between normal responders and individuals who faint.

This study is indicative that the renin-angiotensin system participates in the acute response to postural change in normal man and that it functions abnormally in vasovagal syncope. That the system is not the sole regulator of hemodynamics in relation to posture is indicated clearly by the ability of some anephric individuals to tolerate the upright position. It is likely that neurogenic mechanisms and the catecholamines are a major contributory factor in vasomotor regulation under these circumstances.

ALDOSTERONE

It is evident that experiments in which an attempt was made to define factors regulating vasomotor tone would be more meaningful (and certainly more inclusive) if the renin-angiotensin effector loop could be closed by the inclusion of plasma-aldosterone measurements. Aldosterone is present in normal man in a concentration of only 50 pg/ml (ref. 7-6). Double-isotope-derivative methods for blood necessitate the use of such large sample volumes that the physiological experiment is perturbed by reduction of blood volume by exsanguination, and repetitive measurements are impossible. An unextracted immunoassay would be desirable, but the similarity of the structure of aldosterone to other circulating steroids present in far higher concentration in blood (cortisol and corticosterone, fig. 7-7) has necessitated the combination of chromatographic separations with immunoassay. A simple, direct immunoassay is considered essential in the effective pursuit of physiological studies in man and development has been sought of a test that would avoid all prior chromatography or concentration and that uses minimal volumes of blood.

It has been shown previously that steroid antibodies are capable of having great specificity. Figure 7-8 is indicative that an antibody to digoxin binds digitoxin considerably less well (by a factor of 50), although the two compounds differ only by a single hydroxyl group at position 12 on the steroid ring (ref. 7-7). Conversely, digoxigenin, which differs from digoxin by loss of three digitoxose sugars, binds to antibody nearly as well as digoxin. The reason why a small change in the molecule is recognized in one instance and a larger change is not recognized in the other instance is explained by the structure of the immunogen. Antibody to digoxin is raised by coupling the sugar end of the molecule to a protein carrier and by injecting animals with this protein-steroid conjugate. Antibodies are elaborated that have greatest specificity for the portions of the molecule distal to the carrier protein (the steroid-lactone) and least specificity for those parts nearest the carrier (the sugars).

A study of the structure of aldosterone and similar compounds in figure 7-7 is clearly indicative that antibodies likely to have sufficient specificity for differentiating aldosterone from other circulating steroids must have their major specificity directed toward structures in the D-ring. The most logical site of coupling is position 3. This coupling was accomplished by forming a steroid-hydrazone with aldosterone under conditions such that the Δ^4-3 ketone preferentially reacted rather than the C_{20} ketone (ref. 7-8). The general outline for synthesis of the immunogen is shown in figure 7-9. Analysis of the resulting compound was indicative that a unique substitution at position 3 had occurred. Relative displacement by various steroids for an antibody produced by immunization with this antigen is shown in figure 7-10; 25 pg of unlabeled aldosterone resulted in 15-percent displacement of label, a sensitivity suitable for direct plasma assay. However, cortisol and corticosterone, steroids of very similar structure, required a 10 000-fold or greater concentration for equal displacement. Other steroid compounds were even less cross-reactive.

CONCLUSIONS

The availability of antibodies of such specificity and affinity soon will permit the development of sensitive, direct radioimmunoassays. It is hoped that future examination of postural reflexes in man will include serial blood-aldosterone determinations as well as renin and angiotensin II determinations. Improved analytic techniques for studying hormonal vasoregulation will result in understanding controlling these reflexes that are so important to maintenance of maximal efficiency during altered gravity states.

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TABLE 7-I.- CLASSIFICATION OF SUBJECTS AND THEIR RESPONSE TO TILTING

[From ref. 7-5]

Class of subjects	Normal response	Faint	Total
Normal volunteers	16	4	20
Catheterization patients	7	4	11
Anephrics	4	1	5
Totals	27	9	36

TABLE 7-II.- MEAN RENIN ACTIVITY IN CARDIAC-CATHETERIZATION PATIENTS IN RESPONSE TO TILTING

[From ref. 7-5; results are expressed as ng angiotensin I generated/ml plasma/hr]

Sample source	Resting	Time upright, min					Time supine, min				
		1	3	5	10	20	1	3	5	10	20
Normal responders (n = 7)											
Renal vein	1.64	1.99	2.08	3.05	4.18	5.49	5.49	3.39	3.74	4.37	2.70
Superior vena cava	1.02	1.08	1.38	2.13	1.67	3.46	4.77	2.13	2.62	2.95	2.96
Fainters (n = 4)											
Renal vein	3.82	5.24	4.45	3.78	--	--	13.56	18.9	8.04	7.05	6.70
Superior vena cava	2.08	4.68	1.05	0.98	--	--	5.43	3.21	4.34	4.10	4.29

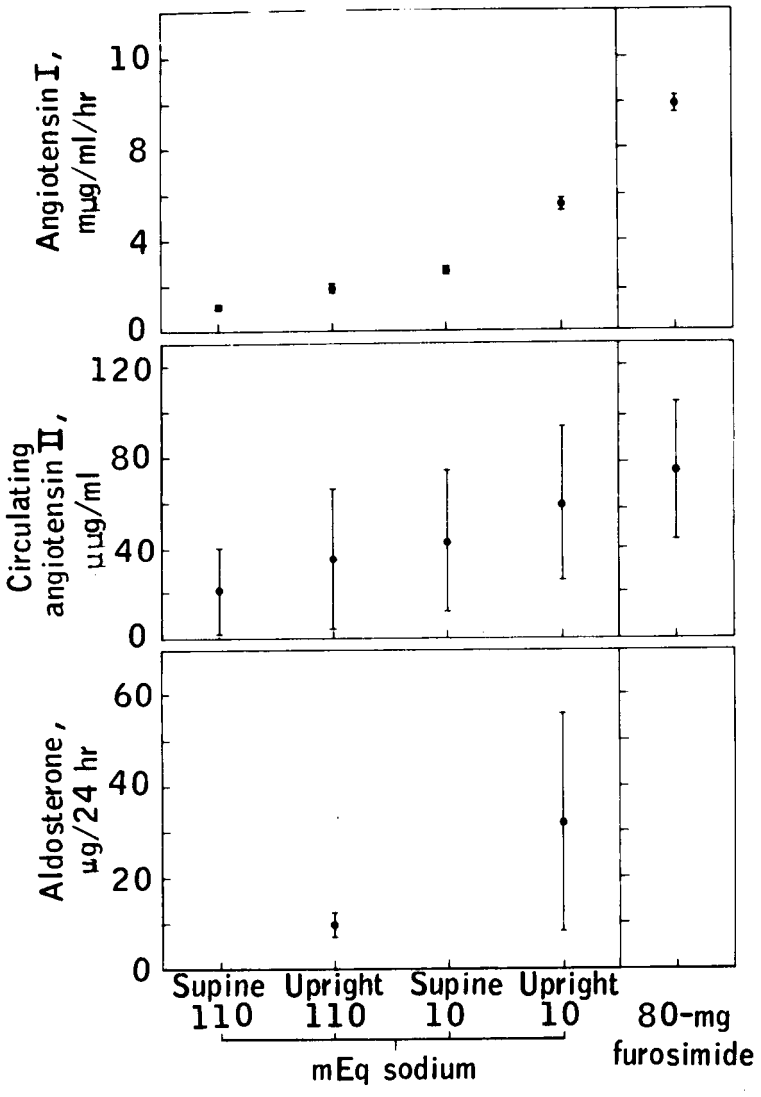


Figure 7-1.- Renin activity measured as angiotensin I produced, venous angiotensin II, and aldosterone excretion in 15 normal subjects under varying "stress" to "effective blood volume." Sodium values represent intake per 24 hours (from ref. 7-3).

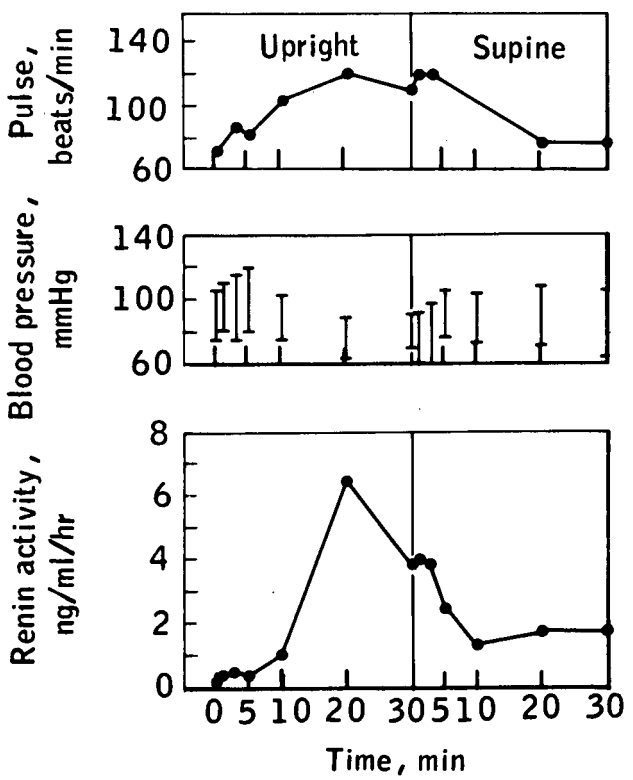


Figure 7-2.- Typical response to tilting in a normal subject who did not faint. The vertical line indicates when supine posture was resumed.

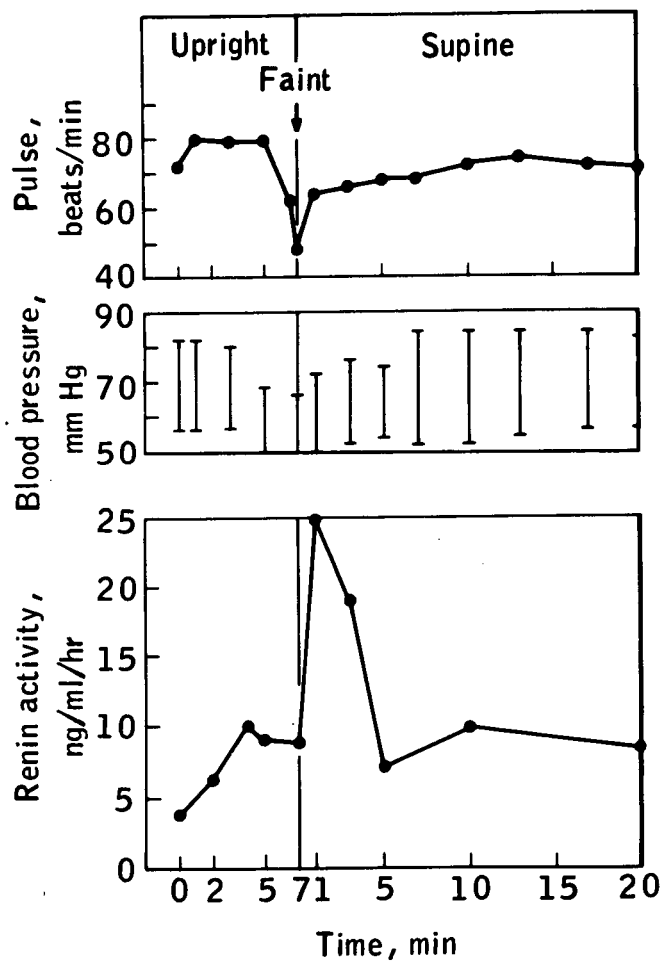


Figure 7-3.- Typical response to tilting in a subject who fainted. Subject placed supine at 7 minutes.

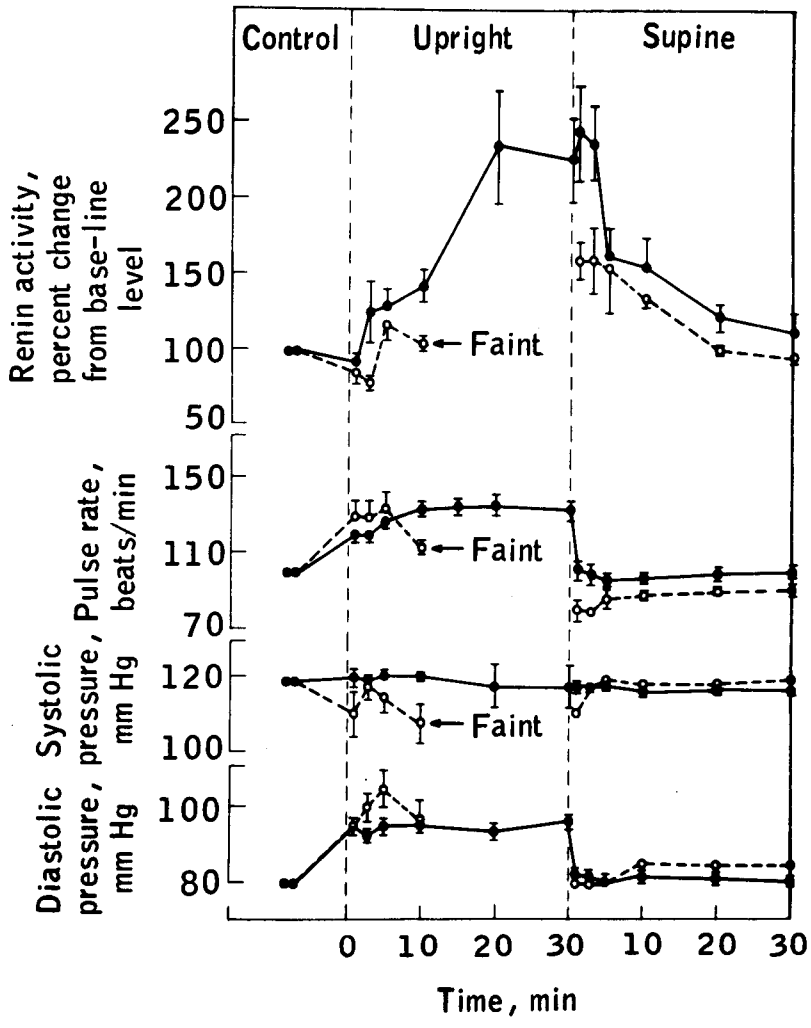


Figure 7-4.- Mean renin activity, pulse rate, and systolic and diastolic blood pressure ± standard error of the mean for the normal volunteers are plotted against time. Values for normal responders are represented by closed circles connected by solid lines; for fainters, by open circles connected by dashed lines. All values are expressed as percent of base line (from ref. 7-5).

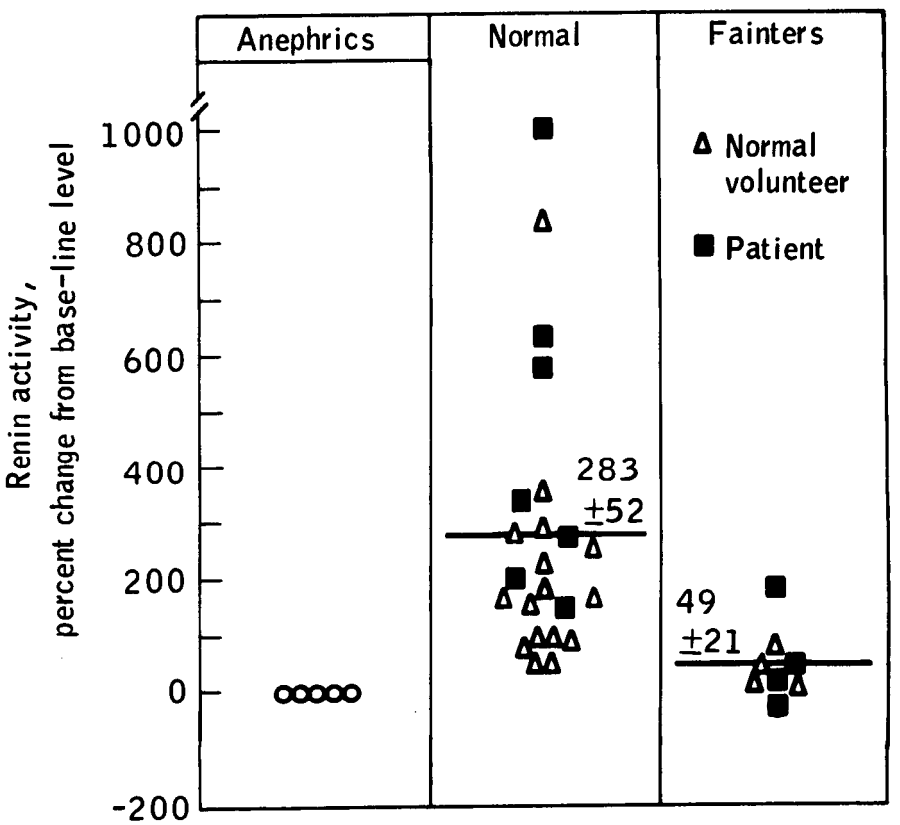


Figure 7-5.- Maximum renin activity following upright tilting for all subjects studied. Each renin value is represented as percentage change from base-line level for the particular subject; figures in each column refer to the mean ± standard error. The means were significantly different ($p < 0.001$) on t-test (from ref. 7-5).

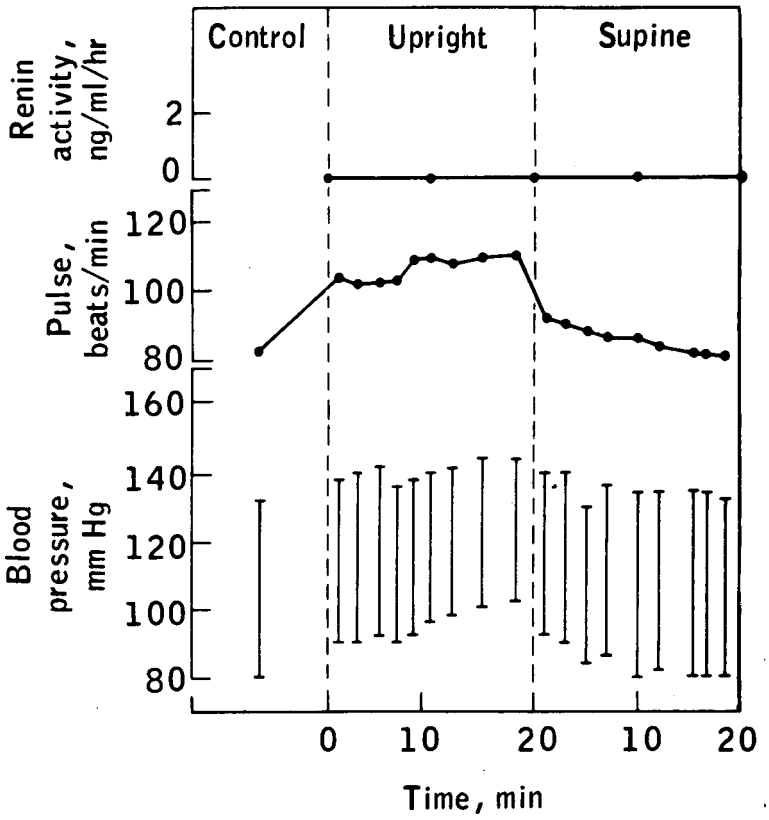
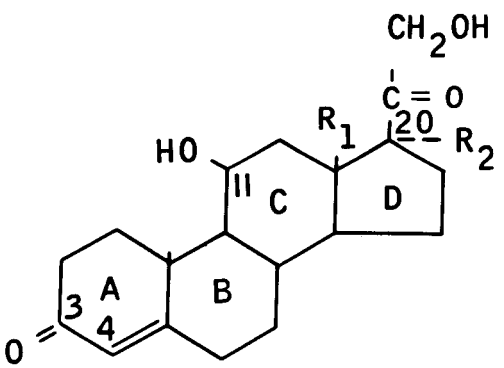


Figure 7-6.- Renin activity, pulse rate, and blood pressure of the normotensive anephric patient are plotted against time (from ref. 7-5).



Steroid	R_1	R_2
Aldosterone	-CHO	-H
Corticosterone	$-CH_3$	-H
Cortisol	$-CH_3$	-OH

Figure 7-7.- Structures of aldosterone, corticosterone, and cortisol. The C_{18} (R_1) aldehyde of aldosterone exists in solution in a hemiacetal link with the C_{11} (from ref. 7-8).

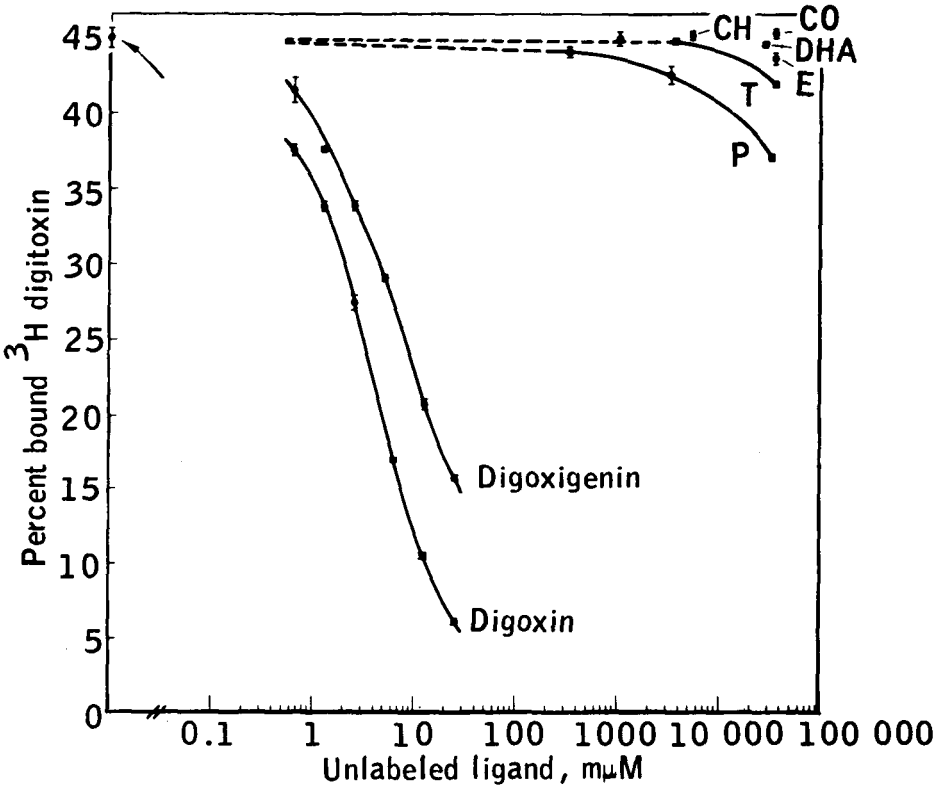


Figure 7-8.- Hapten-inhibition curves (antiserum 46/97) for the steroid compounds digoxigenin, cholesterol (CH), cortisol (CO), dehydroepiandrosterone (DHA), 17 β-estradiol (E), testosterone (T), and progesterone (P), compared with the homologous hapten digoxin. The value for cholesterol represents the concentration at saturation of the aqueous buffer. The arrow on the ordinate denotes binding in the absence of unlabeled ligand. Individual values plotted are means, with ranges of duplicate determinations as shown (from ref. 7-7).

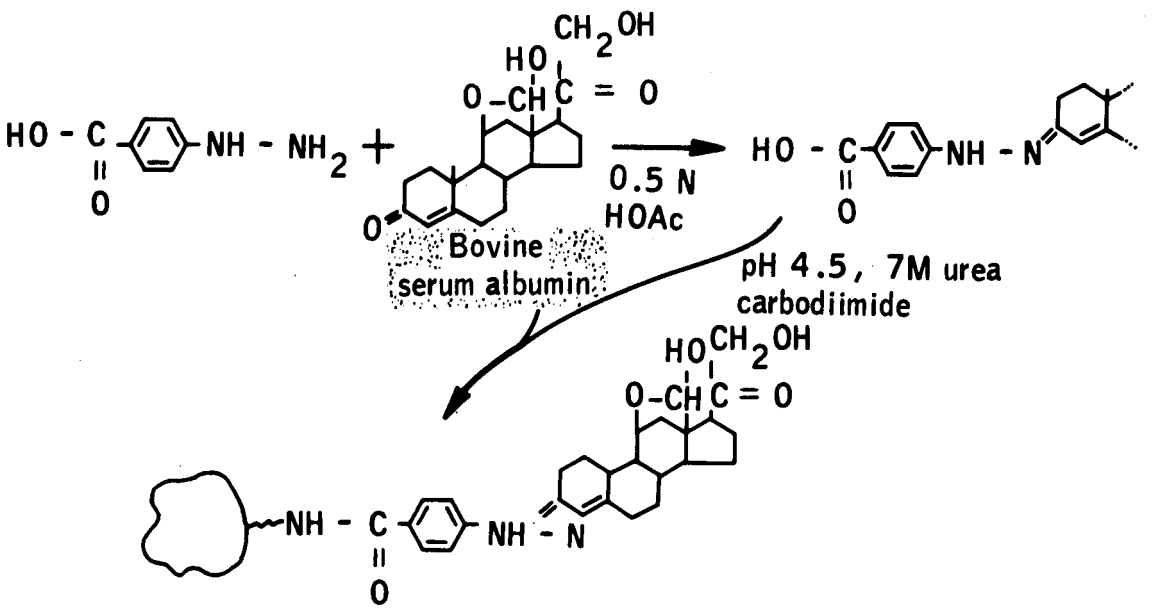


Figure 7-9.- A scheme for synthesis of aldosterone Δ^4-3 hydrazino-benzoic acid-bovine serum albumin.

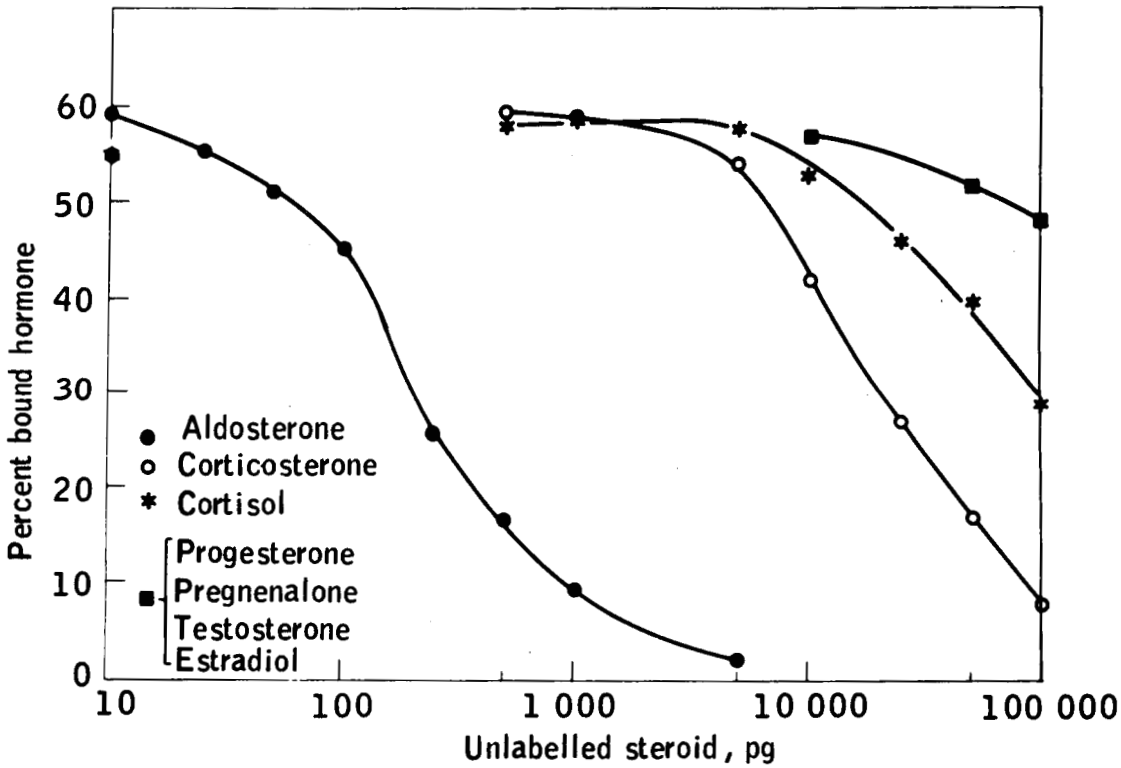


Figure 7-10.- Displacement of ^{3}H-aldosterone by ^{1}H-aldosterone and by other steroids. Incubation mixtures contained 21 pg of ^{3}H-aldosterone and a 1:50 dilution of serum from a rabbit, obtained 10 weeks after initial immunization. In the absence of unlabeled steroids, 61 percent of the ^{3}H-aldosterone was bound by the antiserum (from ref. 7-8).

8. HORMONAL CONTROL OF CALCIUM METABOLISM

By John T. Potts, Jr., M.D.*

INTRODUCTION

Three analytical techniques are of particular interest in the study of calcium and bone metabolism; these techniques are the assays for parathyroid hormone, calcitonin, and vitamin D. Discussion will be limited to the current methods of assay and the uses to which the assays might be put.

DISCUSSION

The parathyroid hormone (PTH) radioimmunoassay has been used for a number of years. Methods have been developed in several independent laboratories, and the assay is proving to be very useful (refs. 8-1 to 8-6). A typical assay result is shown in figure 8-1. The PTH concentrations can be detected in all normal patients; however, patients with both primary and secondary disorders of the parathyroid function usually have much higher PTH assay levels. It has also become evident that, as these tests are applied to define more closely the abnormality in hyperparathyroidism, problems appear. The range of PTH values found in normal subjects in the immunoassay (expressed in terms of a standard preparation of human PTH) average 0.6 to 0.7 ng/ml. The PTH values found during the resting state for a number of patients with primary hyperparathyroidism are shown in figure 8-1. As can be seen, most PTH values are much higher than those found in normal individuals, but an overlap definitely exists. This overlap in PTH values is minimized if a correction is made for basal blood calcium (fig. 8-1). Of all those working with the immunoassay technique, only Reiss and Canterbury (ref. 8-3) have indicated that no overlap exists. The reasons for this will be discussed later. The disagreement may be intrinsic to the problem of the nature of immunoreactive hormone rather than to any assay technique. It would seem that different investigators are measuring different fractions of hormone that is not behaving as a single component in the blood.

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The problem becomes important in the aerospace application of the procedure, both from the viewpoint of improving the accuracy of the diagnostic test for distinguishing clearly normal from abnormal functioning and, in the case of men exposed to weightlessness, for establishing very clearly the limits of abnormal functioning. The problem of overlapping values, which may in part be caused by immunochemical heterogeneity, will have to be solved.

When a parathyroid adenoma is removed from a patient with primary hyperparathyroidism, the expectation is, on the basis of earlier investigations in which animals were used (ref. 8-7), to see the hormone concentration decrease very sharply in a monotonic fashion to undetectable or very low levels. In each of 20 patients from whom a parathyroid adenoma had been removed, a rapid decrease in hormone concentration was noted initially, which indicated the removal of the source of hypersection. However, a flattening out in the rate of decrease of hormone concentration occurred and was followed by the disappearance of the second component(s) from blood with a much slower half-time (T 1/2). The initial T 1/2 is on the order of approximately 15 to 18 minutes, and the second T 1/2 is several hours (fig. 8-2).

The problem is even worse for patients with chronic renal failure (fig. 8-3), who often have increased PTH levels because of secondary hyperparathyroidism. The data points indicated by filled circles represent the concentration of immunoassayable hormone in the blood for a patient with chronic renal failure and from whom all four parathyroids were removed at the judgment of the patient's physicians because of severe bone disease and other complications. The data points indicated by open circles represent the patient's serum calcium, which was at a preoperative level of 10.5 milligram-percent. Approximately 100 minutes after the parathyroidectomy was performed, an abrupt decrease of the patient's calcium concentration to tetanic levels was observed. Yet, the hormone was disappearing from the blood very slowly, with no fast component at all. At the time the patient experienced symptoms of tetany, the immunoassayable hormone in the blood was still present in the hyperparathyroid range.

An even more severe example (fig. 8-4) is taken from some stimulation and suppression tests in another patient with chronic renal failure (in this case, immediately after successful renal transplantation). When calcium was infused (fig. 8-4(a)) to raise the calcium level from approximately 10 to 14 milligram-percent, hardly any change in the immunoassayable-hormone concentration in the blood resulted. However, when an ethylenediaminetetracetic acid (EDTA) infusion was given (fig. 8-4(b)) to lower the ionized calcium and to stimulate the hyperplastic parathyroid glands, a sudden rise in the immunoassayable-hormone concentration was observed. This phenomenon seems to indicate that, at any one time, a pool of material is present in the blood that consists of at least

two components, both of which are being measured. One component is behaving as though it is hormone recently released from the gland, disappearing from blood quickly when gland-secretory activity is suppressed or parathyroid tissue is removed. Another component, which is present in variable concentrations, has a very slow metabolic clearance. In the chronic-renal-failure patient who is treated with EDTA, it is very easy to witness an increase in the total pool. However, because, under basal conditions, the concentration of the rapidly cleared material is quite small in relation to the total pool of immunoreactive hormone, it is difficult to detect a decrease, even when new hormone release is abolished. This phenomenon does not mean that the assay is detecting nonparathyroid-related peptides. In patients without parathyroid glands, no immunoreactive hormone is detected. If a patient's hormone level is observed after a parathyroid adenoma is removed, it will be noted that, after an initial rapid fall, the hormone level decreases slowly to normal or undetectable levels after a period of days. Berson (ref. 8-8) has discussed this overall phenomenon, and the problem of immunoreactive and metabolic heterogeneity of plasma parathyroid hormone is being studied by many investigators. The problem was the subject of a recent review at the Mayo Clinic in Rochester, Minnesota (ref. 8-4), at which similar phenomena were discussed.

Of additional interest is the finding that parathyroid adenomas are not at all fixed and autonomous in their hormone output, with respect to either stimulation or suppression (ref. 8-4). A typical example of patients with a parathyroid adenoma (proven at surgery) who had been given EDTA stimulation is shown in figure 8-5. A very brisk rise which approximates the proportional type of control that is seen in normal subjects, is observed in immunoassayable-hormone concentration. It is presumed that some portion of the hormone detected in the basal state corresponds to that observed in the patient who has chronic renal failure; that is, the material consists of one component that disappears slowly and another component that consists of hormone recently released from the gland. It is believed that what is observed is the increase of recently secreted hormone after EDTA stimulation. A more extreme phenomenon is observed in the suppression tests (fig. 8-6). Many patients with parathyroid adenomas who were given a calcium infusion experienced a brisk, but only partial, suppression of hormone concentration. Again, the assumption is made that the observed phenomenon does not indicate incomplete suppression but rather an immediate cessation of output from the gland, followed by a gradual decrease in the concentration of slowly metabolized material. The slowly metabolized material has a very slow metabolic rate, analogous to the persistence of immunoreactive hormone material with a slow disappearance T 1/2 in patients from whom abnormal parathyroid glands have been removed. In patients with chief-cell hyperplasia, the hormone concentration is also suppressed. This instance is the second for which findings are in apparent disagreement with those of Reiss and Canterbury

(ref. 8-3), who have observed that the adenomas invariably produce a completely fixed, constant output of hormone, but that the output of hyperplastic glands can be suppressed.

One other point of interest is that very useful information has been gained from the EDTA tests, even though these tests are not helpful for testing autonomy. It has been learned that the EDTA challenge test can be used as an approximate indication of the amount of parathyroid tissue present. The response, in terms of the rise in the immunoassayable-hormone concentration, is closely related to the size of tissue found subsequently at surgery. For example, the hormone level of a patient with a small adenoma and whose level rose from 0.7 to 1.5 ng/ml with EDTA stimulation is shown in figure 8-7(a). These data can be contrasted to the changes that occurred in another patient whose initial hormone concentration was 3 ng/ml and rose to 17 ng/ml (fig. 8-7(b)). This contrast is made more obvious in figure 8-8, in which the responses in the two patients are plotted on the same scale. The magnitude of the response in the second patient is so enormous as to make the response of the first patient seem negligible. Thus, some insight into parathyroid reserve and into the degree of enlargement of the parathyroids can perhaps be gained by the use of these test methods.

The test data exemplify the types of situations that are presently being studied. Despite the inherent difficulties in the assay, it appears that the greater the mass of abnormal parathyroid tissue and, in general, the more severe the hyperparathyroidism, the greater the basal concentrations of hormone will be, compared with hormone-concentration values found in normal subjects. The EDTA provocation test is particularly useful, because decisions about the existence of abnormalities in glandular function can be made much more easily if measurements are made of the amount of hormone that is present in the blood on the extreme of the response curve, at which point the difference between normal and abnormal is magnified.

The meaning of the observations concerning immunochemical heterogeneity has not been clearly established. It seems likely that what is being measured as PTH in the blood is the result of a combination of the secretion of newly biosynthesized hormone and the metabolic degradation of the hormone after release from the gland. The hormone released from the gland is cleared perhaps through enzymatic digestion, but the ultimate disposal of some of these cleavage products or fragments may be a relatively slow step. Fragments may be bound to plasma protein, may be circulated in the blood as self-aggregates, or simply may be cleared from the blood very slowly. The persistence of some of this material interferes with assessment of the secretory activity of the gland after suppression by calcium infusion.

A resolution of the explanation for the heterogeneity of plasma PTH, however, now seems quite possible. Recent developments, with respect to the structure and synthesis of PTH, have opened the way to resolving the chemical nature of the various forms of circulating PTH. The complete amino-acid sequence of the bovine hormone has been determined (fig. 8-9), and the amino-acid sequence of the porcine hormone has also been determined (fig. 8-10). The amino-acid sequence in the amino terminal region is the sequence that is important for biological activity of the hormones. Using the technique of solid-phase peptide synthesis, Dr. G. W. Tregear has just completed a successful sequence of the 1-to-34 sequence of PTH and has produced what is believed to be a preparation of synthetic hormone that is highly active both in vivo and in vitro on bone and kidney. This synthesis of hormone fragments should make possible systematic examination of the nature of the circulating fragments of hormone by using different region-specific immunoassays that are prepared by immunization with selected sequences of the hormone. In this way, the problem of immunochemical heterogeneity can be approached directly, and decisions can be made as to whether the material that slowly disappears from the blood represents a specific portion of the amino-acid sequence. Then, that portion of the sequence could be measured or not measured, depending on which antiserum is used in the assay system. Certain portions of PTH have already been synthesized for the purpose of making fragment-specific antibodies; namely, the 1-to-13, 14-to-34, 19-to-34, and 25-to-34 sequences. Thus, in addition to making the active fragment for biological work, the sequence is being completed region by region for use in the immunoassay study.

Calcitonin Assay

Various calcitonins from several different species have been isolated, and the structures have been determined (ref. 8-9). Although the salmon hormone is perhaps the most interesting from a therapeutic point of view (ref. 8-10), it is a human-calcitonin assay that is of primary interest from a physiological point of view. Recently, Dr. L. J. Deftos has been successful in developing a very sensitive radioimmunoassay for human calcitonin (refs. 8-11 to 8-13). In contrast to the PTH assay, no cross-reactivity occurs between human and animal calcitonins. Therefore, although specific immunoassays for salmon, bovine, porcine, and ovine calcitonin existed, none was of any use in measuring the human hormone. A very sensitive immunoassay based on antihuman calcitonin antisera and a small amount of the synthetic human hormone was therefore developed. This immunoassay, which can be used to detect as little as 10 pg/ml of calcitonin (fig. 8-11), has proved quite suitable for detection of the calcitonin concentrations in patients with medullary carcinoma. As indicated on the logarithmic scale, these patients have peripheral concentrations of calcitonin as high as 100 000 pg/ml, in contrast to the

normal group of patients, most of whom have calcitonin concentrations that are generally undetectable. Despite this contrast, it has been possible to begin studying the dynamics of calcitonin in man by studying patients with medullary thyroid carcinoma. For example, as shown in figure 8-12, a calcium infusion in a patient with medullary thyroid carcinoma results in a marked and very prompt rise in immunoassayable calcitonin. Two different rates of calcium infusion result — a modest increase and a very large increase in calcitonin. As shown in figure 8-13, the tumor is responsive in both directions; that is, an EDTA infusion to lower the calcium in the blood will cause a suppression of hormone production. Glucagon has a variable effect on immunoassayable calcitonin in humans. With improved techniques, it is hoped that the assay can be used as a tool to study adaptation of hormone secretion to skeletal stress (e.g., whether calcitonin secretion increases directly or indirectly in response to increased bone resorption).

Vitamin D Assay

DeLuca et alii (ref. 8-14) have revolutionized the area of vitamin D study over the last 3 or 4 years with observations that support the view that vitamin D is, in many ways, more like a hormone than a vitamin. It is derived either from the diet or endogeneously from the skin where an inactive precursor, dehydrocholesterol, is converted to vitamin D by irradiation from the sun. Vitamin D, however, is inactive and requires conversion to 25-OH vitamin D by a specific hydroxylase in the liver to exert its physiologic action. It would appear that dihydrotachysterol and ergocalciferol also undergo such metabolic conversion with specific hydroxylation at position 25 on the side chain. Furthermore, DeLuca et alii (refs. 8-15 and 8-16) have shown that, subsequent to this process, further hydroxylation occurs in other tissues and that the final active products are probably dihydroxy metabolites of vitamin D. In any study of the results of chronic environmental stress or various disease states in man, it now seems that vitamin D metabolism, which is ordinarily closely regulated, is subject to control at multiple points and, therefore, has many points at which derangement in control may occur. The result of prolonged sunlight deprivation and inadequate vitamin D synthesis or interference with the efficiency of conversion of the D_3 to the 25-OH compound remains to be seen. These areas constitute a very important field for investigation. What has been lacking is a sensitive method for chemically measuring 25-OH vitamin D and vitamin D directly. Fortunately, Dr. R. Belsey has been successful in applying a Murphy-type saturation-analysis technique to the detection of vitamin D at the physiological levels (ref. 8-17). At the present time, a simple extractive and chromatographic step that separates vitamin D_3 from 25-OH vitamin D_3 is used. Because both compounds are bound to the specific vitamin-D-binding protein, measurement of the physiological levels of both compounds

is possible (fig. 8-14). Evidently, in man, several vitamin-D-binding proteins exist, one of which binds vitamin D preferentially while the other preferentially binds 25-OH vitamin D. Thus, it is possible that two specific ligand systems could be developed for direct determination of both vitamin D and 25-OH vitamin D without a chromatographic step, if the separate vitamin-D-binding proteins can be isolated.

At the present time, the binding protein from blood has been useful for detection of vitamin D by means of the usual competition between tritium-labeled vitamin D and added unlabeled material. The assayable quantity can be as minute as 1 nanogram of vitamin D_3, which is well below the physiological range. This technique can now be applied in a series of studies of vitamin D and its active metabolites in various disease states and — of particular interest in the aerospace studies — under various physiological adaptation situations (such as the skeletal stress induced by weightlessness). It will be important to determine the rate of decline in blood levels of vitamin D and 25-OH vitamin D in the total absence of sunlight or ultraviolet irradiation, as in prolonged space flight.

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8-17. Belsey, R.; DeLuca, H. F.; and Potts, J. T., Jr.: Competitive Binding Assay for Vitamin D and 25-OH Vitamin D. Proceedings of the 4th Parathyroid Conference Excerpta Medica, 1971.

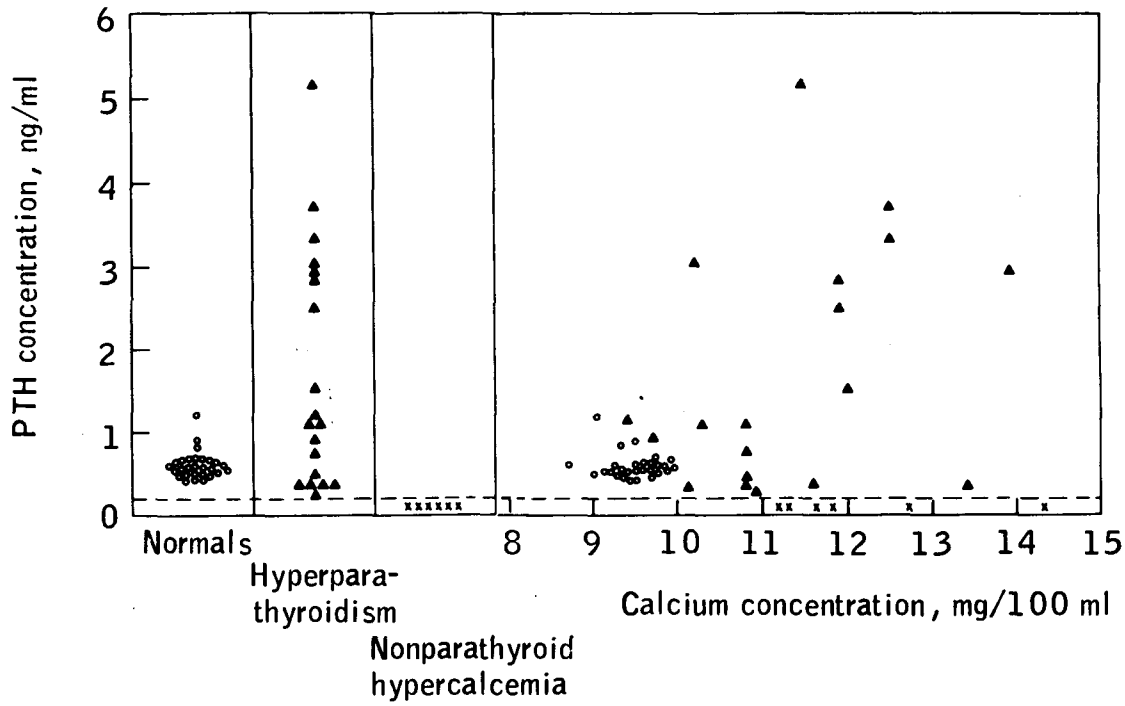


Figure 8-1. Basal fasting-plasma PTH concentration in 30 normal adult subjects (o), 20 patients with primary hyperparathyroidism (▲), and six patients with nonparathyroid hypercalcemia (x). In the right-hand panel, the PTH concentrations are plotted as a function of the basal serum-calcium concentration.

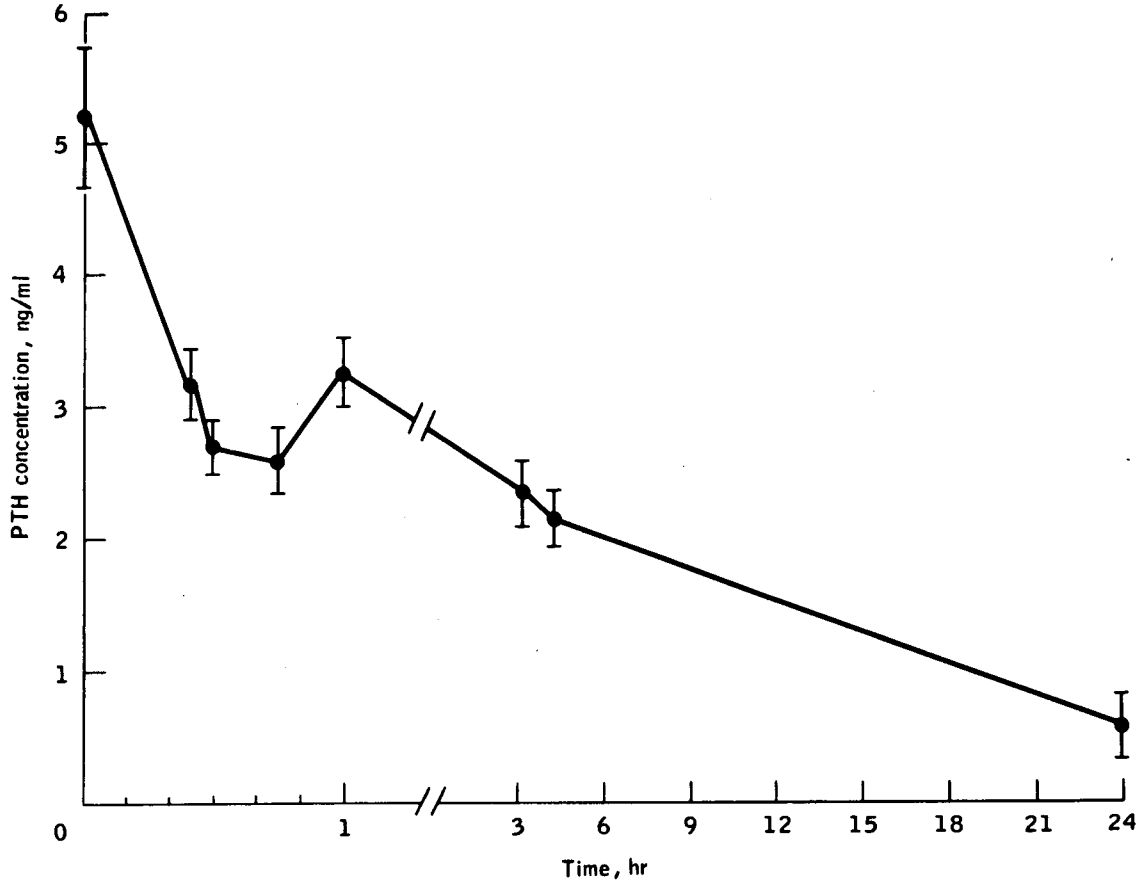


Figure 8-2. Plasma PTH concentration in a patient with primary hyperparathyroidism (as a function of time with T 1/2 indicated) following the removal of a parathyroid adenoma.

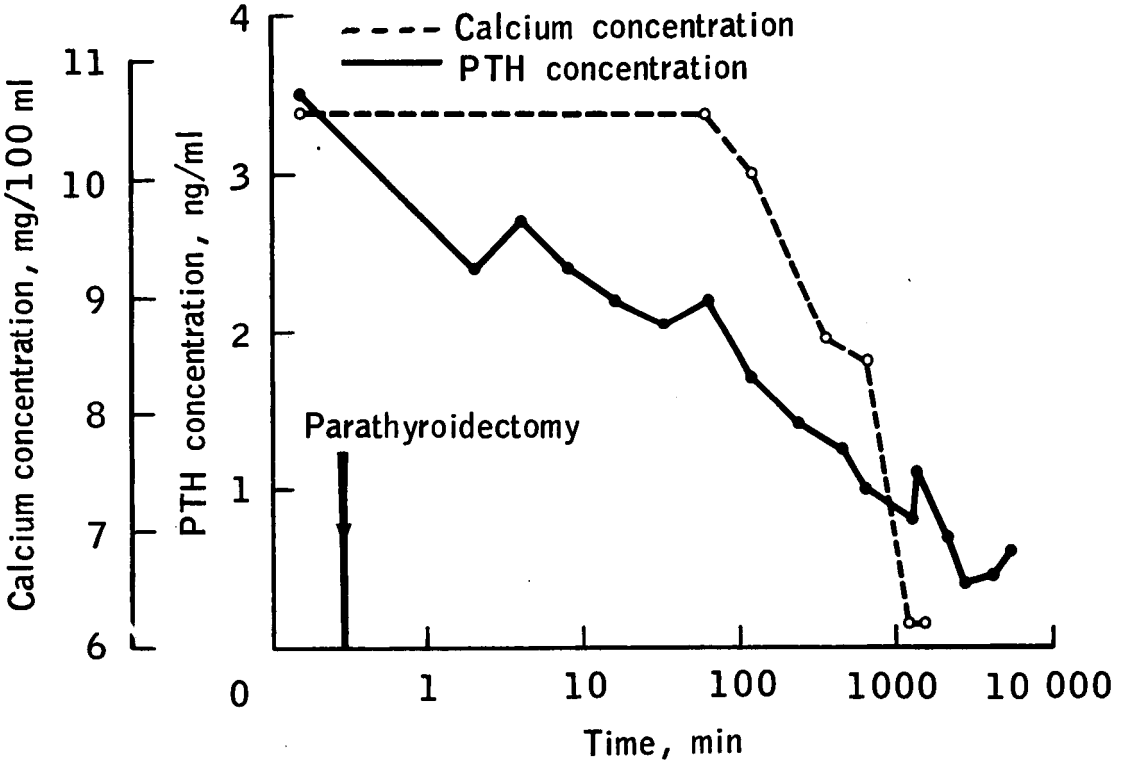
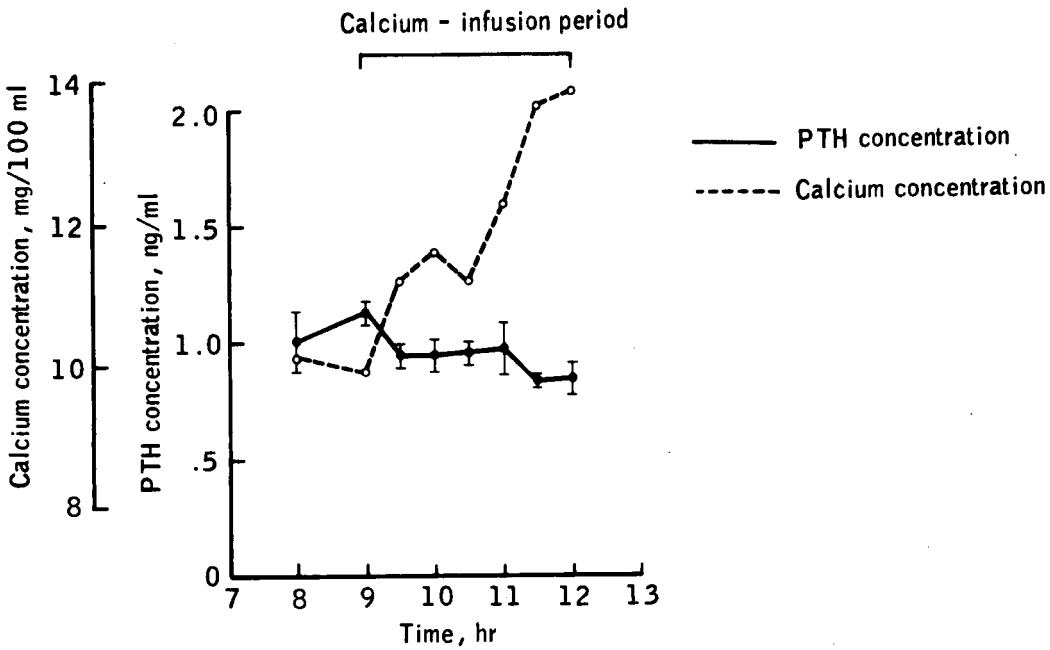
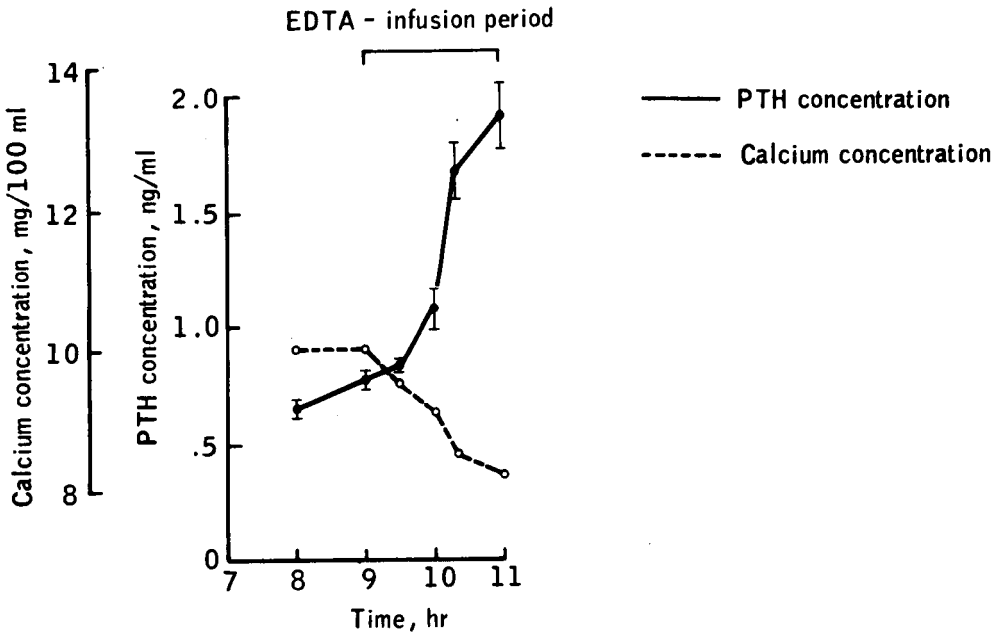


Figure 8-3. Plasma PTH concentration (●) and serum-calcium concentration (o) in a patient with chronic renal failure as a function of time after subtotal parathyroidectomy.

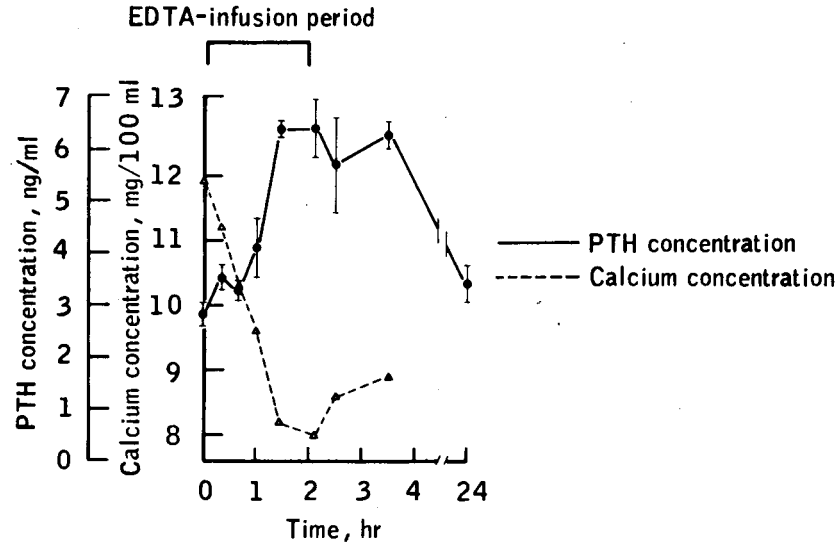


(a) During calcium infusion.

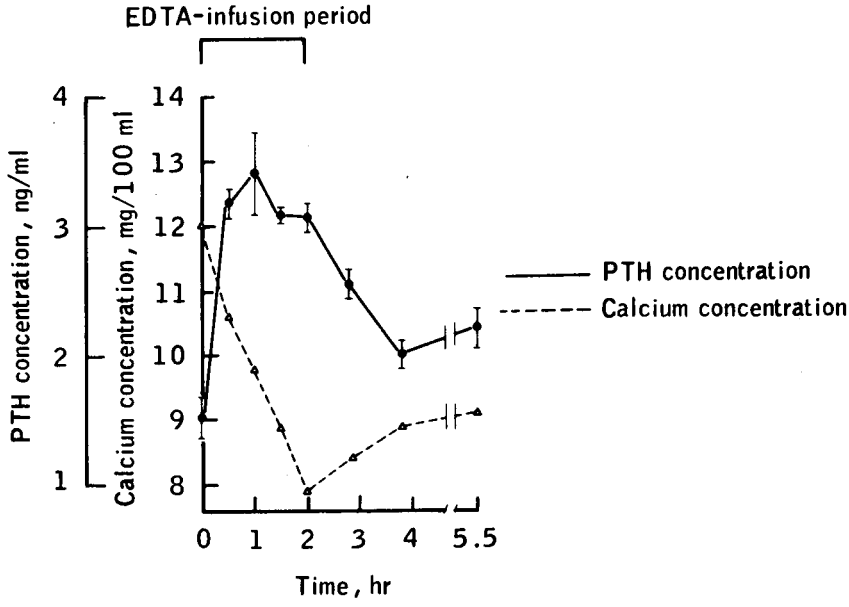


(b) During EDTA infusion.

Figure 8-4. Plasma PTH concentration (●) and serum-calcium concentration (o) in a patient with chronic renal failure.



(a) Patient 1.



(b) Patient 2.

Figure 8-5. Plasma PTH concentration (●) and serum-calcium concentration (Δ) during EDTA infusion in two patients with parathyroid adenomas.

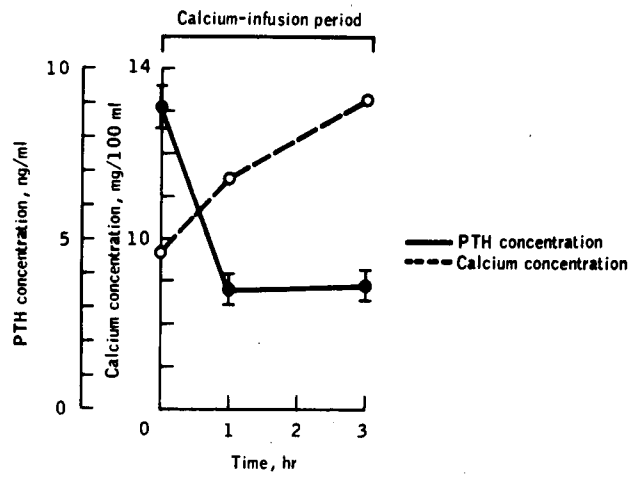
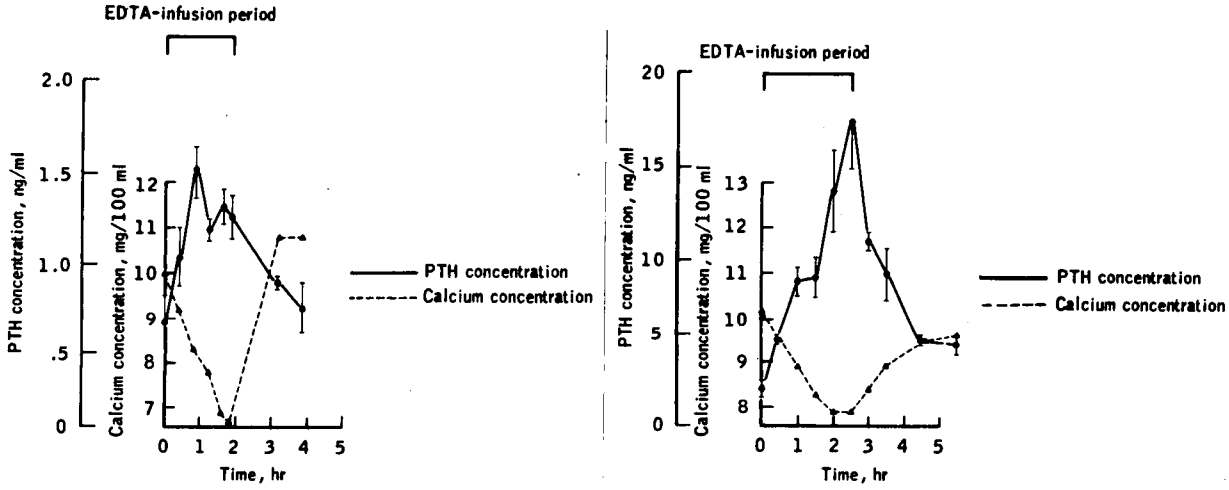


Figure 8-6. Plasma PTH concentration (●) and serum-calcium concentration (o) during calcium infusion in a patient with a parathyroid adenoma.



(a) Patient 1.

(b) Patient 2.

Figure 8-7. Plasma PTH concentration (●) and serum-calcium (Δ) concentration during EDTA infusion in two patients with primary hyperparathyroidism.

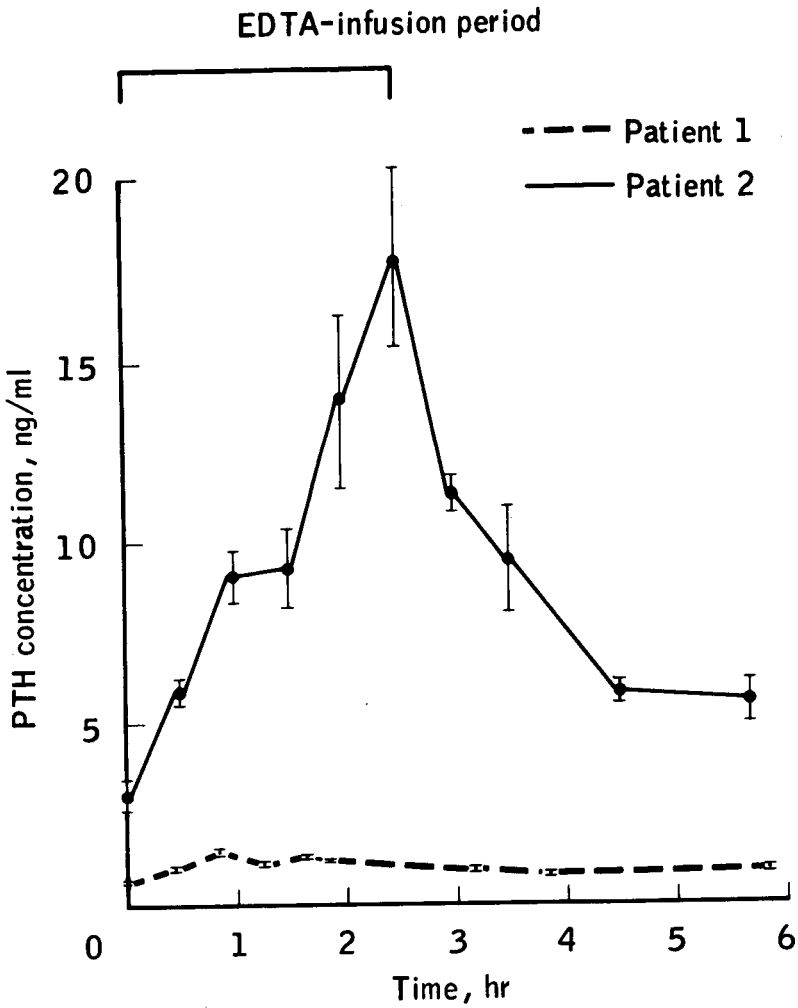


Figure 8-8. The PTH concentrations for patients 1 and 2 from figure 8-7 replotted on the same scale.

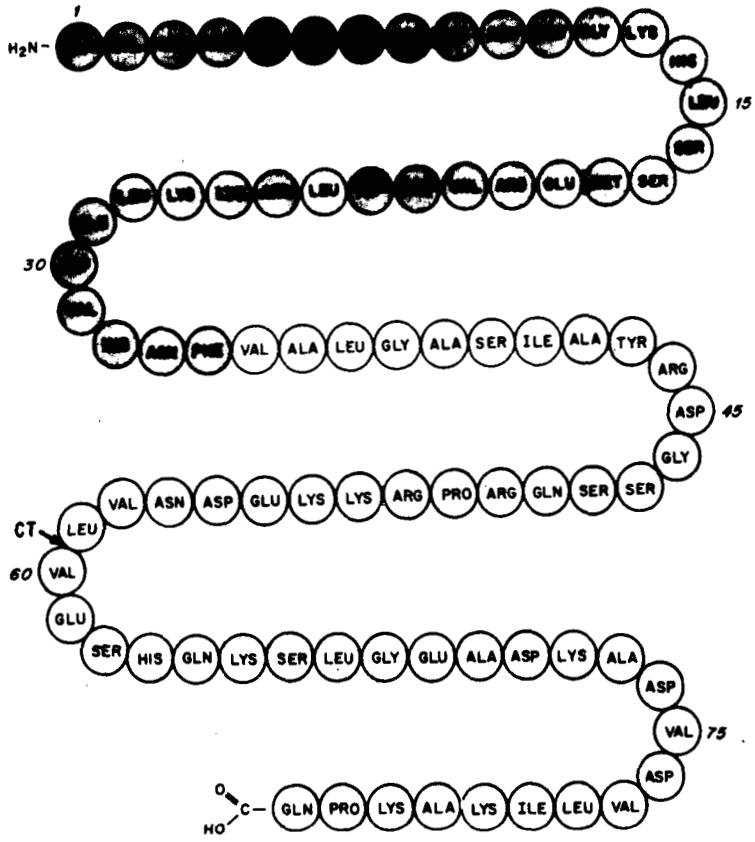


Figure 8-9. Amino-acid sequence of bovine PTH. Shaded residues represent the region of the molecule that exhibits the biological activity of the native molecule.

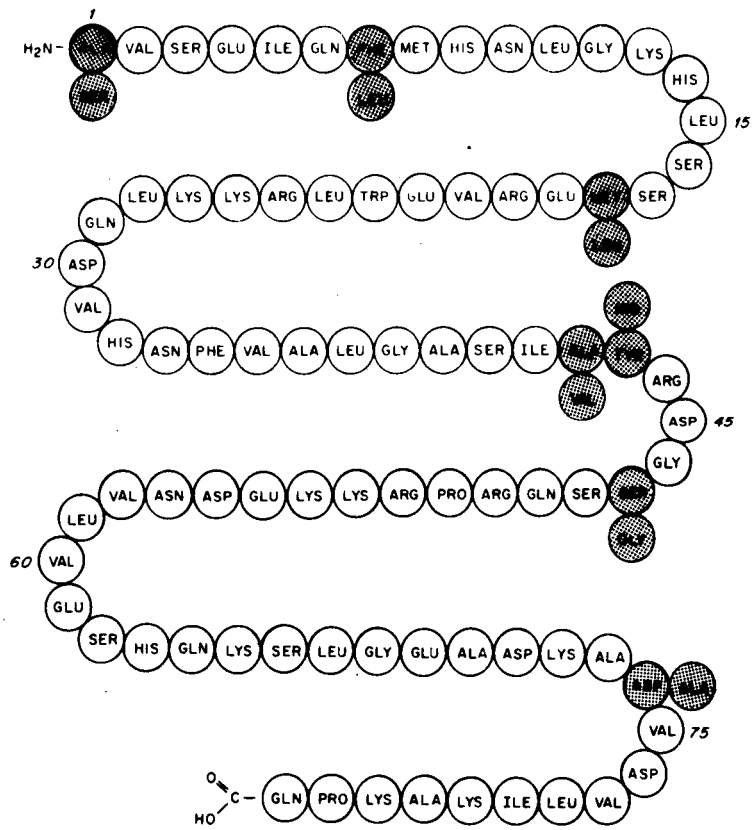


Figure 8-10. The amino-acid sequence of bovine PTH, as compared to the amino-acid sequence of porcine PTH. The differences in the porcine structure are represented by the shaded residues.

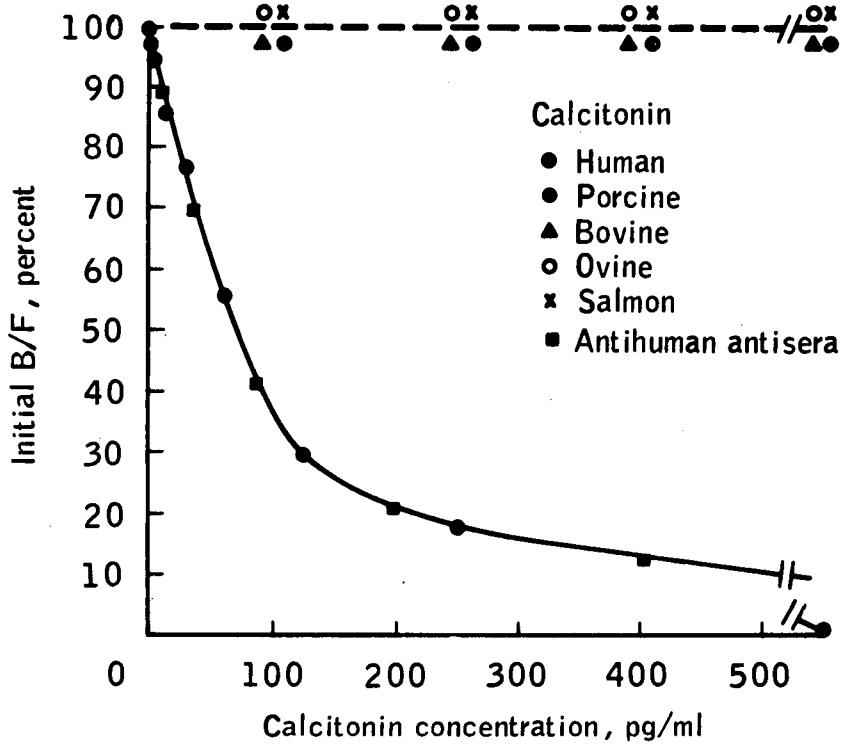
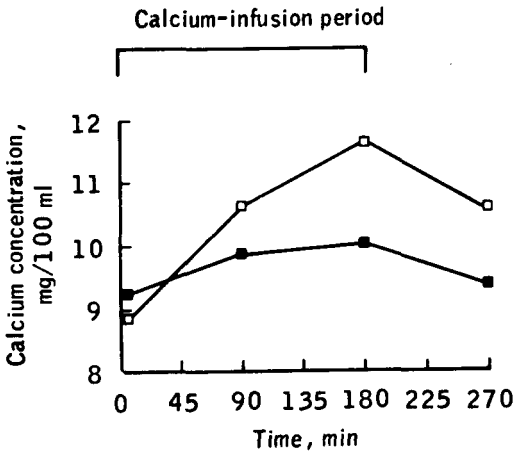
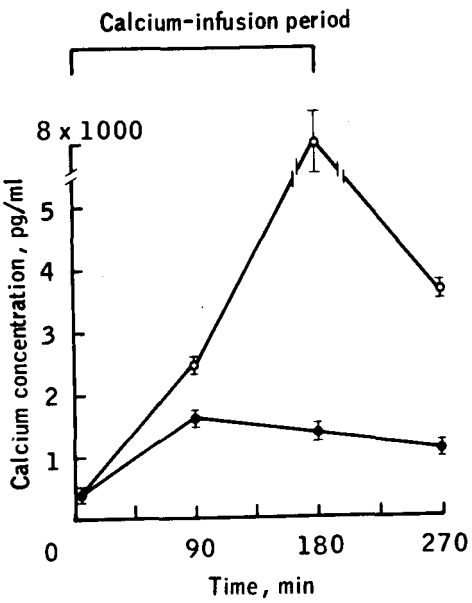


Figure 8-11. Radioimmunoassay for human calcitonin. No cross-reactivity of calcitonin of the other species tested occurs.



(a) Calcium concentration.



(b) Calcitonin concentration.

Figure 8-12. Calcium concentration and calcitonin concentration in a patient with medullary thyroid carcinoma by calcium infusion at two rates, 6 mg/kg/hr (■ and ●) and 12 mg/kg/hr (□ and ○).

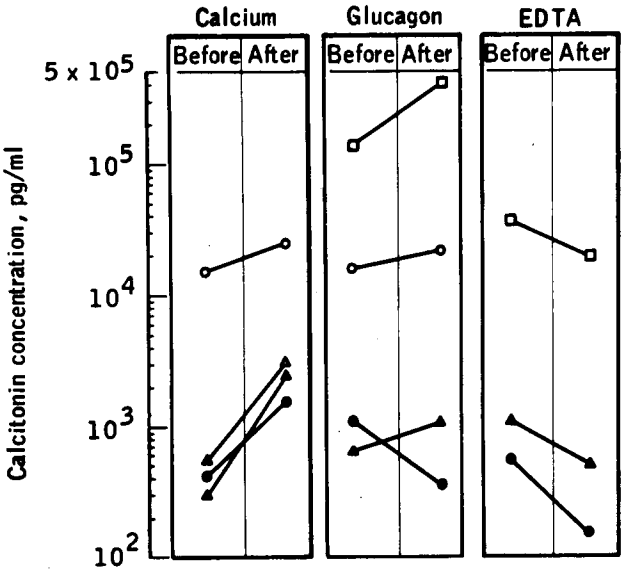


Figure 8-13. Effect of functional tests on the secretion of calcitonin in patients with medullary thyroid carcinoma. The lines connecting symbols indicate changes for different patients.

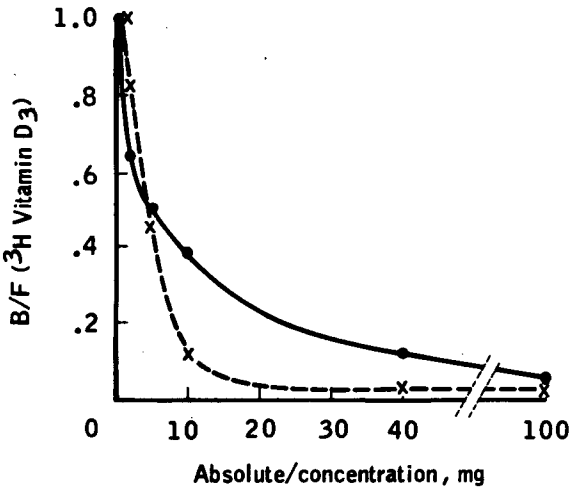


Figure 8-14. Standard curves of competitive binding assay for vitamin D_3 (•) and 25-OH vitamin D_3 (x).

9. PEPTIDE HORMONES IN URINE

By Don H. Nelson, M.D.* and John E. Bethune, M.D.†

INTRODUCTION

The program of studying peptide hormones in urine was initiated as part of an overall investigation of the effects of space flight on bone metabolism as related to a pituitary-adrenal stress and parathyroid hormone (PTH) secretion. Because it was evident that only urine samples would be available (for some time in the future), it was proposed to develop suitable methods for assaying PTH and adrenocorticatrophic hormone (ACTH) in urine as measures of stress and parathyroid function. Therefore, progress in assaying these two peptides will be summarized in this report.

DISCUSSION

Parathyroid hormone has been studied more extensively than ACTH and will be discussed first. Previous studies done in our laboratory have suggested that urine contained a parathyroid-like substance that had biologic properties similar to that of beef PTH. Therefore, it seemed necessary to improve the methods of bioassaying and extracting this material from urine quantitatively, so that differences between control and inflight conditions would be meaningful. A relatively simple bioassay in mice was developed, using thyroparathyrocautery of 8- to 10-gram mice and measurement of whole-blood calcium 5 hours after hormone injection. This procedure has proven to be reliable. The results of a typical assay are shown in figure 9-1, which depicts linearity from 0.03 to 2.0 United States Pharmacopeia (U.S.P.) units of commercial beef PTH. A bioassay procedure was used because it was discovered early that the material with biological activity in urine did not react very well immunochemically with beef or human PTH. In fact, this material has such low immunological activity that using this method as a measurement

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of the peptide activity was impossible. For this reason, it was necessary to study the identity of the material in the urine. This study was performed through a series of experiments in which large amounts of the material in urine were collected and subjected to chromatography and disc-gel electrophoresis. As shown in figure 9-2, the biological activity run on a Sephadex G-50 column is at a rate identical to that of a commercial Dextran preparation with an average molecular weight of 9400. This observation suggested that the molecular weight of the material was in the range expected for PTH. As shown in figures 9-3 and 9-4, the elution patterns of pure beef PTH labeled with radioactive iodine and bioassayable PTH-like activity from patients with uremia (fig. 9-3) and patients with parathyroid adenoma (fig. 9-4) were almost identical. These samples were run on Sephadex G-75 in a 2.5- × 175-cm column and eluted with 0.1 molar sodium acetate. The material from several patients was then collected and chromatographed on a Bio-Gel P-10 column; the eluant was bioassayed, counted for radioactivity, and compared with the average running rates (as shown in the lower portion of fig. 9-5) with cytochrome c with a molecular weight of 12 300, with Glucagon with an average molecular weight of 3485, and with the Dextran D-10 with a molecular weight of 9400. It was concluded that the mobility of this material was similar to that of beef PTH. The material from the effluent of the Bio-Gel column was lyophilized and subjected to a disc-gel electrophoresis (fig. 9-6). Purified beef PTH was applied to gel A, the urinary PTH collected from patients was applied to gel B, and a mixture of beef PTH and the effluent was applied to gel C. The running rates were similar and a single peak was observed in each instance. The chromatographically pure material from patients was then applied to a carboxymethylcellulose column and eluted with an increasing gradient of sodium chloride. Three distinct peaks of protein were observed, two of which had bioassayable activity with specific activities of 500 and approximately 1500 units/mg each.

Currently, work is in progress on collecting material from large amounts of urine in an attempt to identify these peaks further. The two peaks have biological activity sharing a dose-response curve identical to PTH but have 1 percent or less of the immunological activity of an equivalent amount of beef PTH. Therefore, it would seem that an altered PTH is being measured in the urine. The concentration of material in urine correlates with states in which overactivity or underactivity of the parathyroid glands is known to exist. It is believed that parathyroid function is being reliably measured. The technique for measuring this material, as finally evolved, is to take a 24-hour aliquot of urine, add minute amounts of radioiodinated beef hormone, concentrate the material with silica, elute the silica with acid acetone, lyophilize, reconstitute (counting to correct for losses), and inject the material into assay animals. Normal subjects have had less than 40 units/day of PTH in the urine. Currently, an attempt is being made to develop a procedure in which a smaller aliquot of urine would yield reliable results.

It has been known for some time that a polypeptide with ACTH activity is found in urine. It has been shown that the material in urine of patients with Addison's disease and Cushing's syndrome is immunologically reactive; therefore, it has been possible to develop and use an immunoassay for measuring this material. Although results to date are preliminary, a comparison of the bioassayable and immunoassayable ACTH in urine of patients with Cushing's syndrome and in Addison's disease has been made (table 9-I). This comparison suggested that the quantity of immunoassayable material may, in general, be greater than the bioassayable material. The same relationship can be observed to be true for blood ACTH as well. Further work on this needs to be done.

CONCLUDING REMARKS

Difficulty has been experienced in measuring immunoassayable ACTH in urine of normal subjects, because the amount is barely detectable by assay in untreated urine, perhaps as a result of salt concentration variation in urine samples. Attempts to concentrate the material by lyophilization have failed. Currently, studies conducted using silica adsorption have not been completely successful, because reproducibility and recovery rates have not been uniform. Quite likely, minute quantities of radioactive ACTH will have to be added to the urine to make corrections for each assay, following adsorption by silica particles. This technique will be advantageous, because one procedure will be usable for both PTH and ACTH extraction.

TABLE 9-I.- URINARY ACTH

Patient	Bioassay, mU/day	Immunoassay		Diagnosis
		µµg/day	mU/day	
1	14.04	32 500	19.4	Cushing's syndrome
2	6.44	28 000	18.06	Cushing's syndrome
3	6.244	37 250	24.9	Cushing's syndrome
4	3.34	12 750	8.5	Addison's disease
5	--	24 000	16.0	Cushing's syndrome (postadrenalectomy)
6	--	15 350	10.2	Addison's disease
7	--	26 500	17.6	Cushing's syndrome
8		--	--	Cushing's syndrome (preadrenalectomy)
8		7 000	4.6	Postadrenalectomy

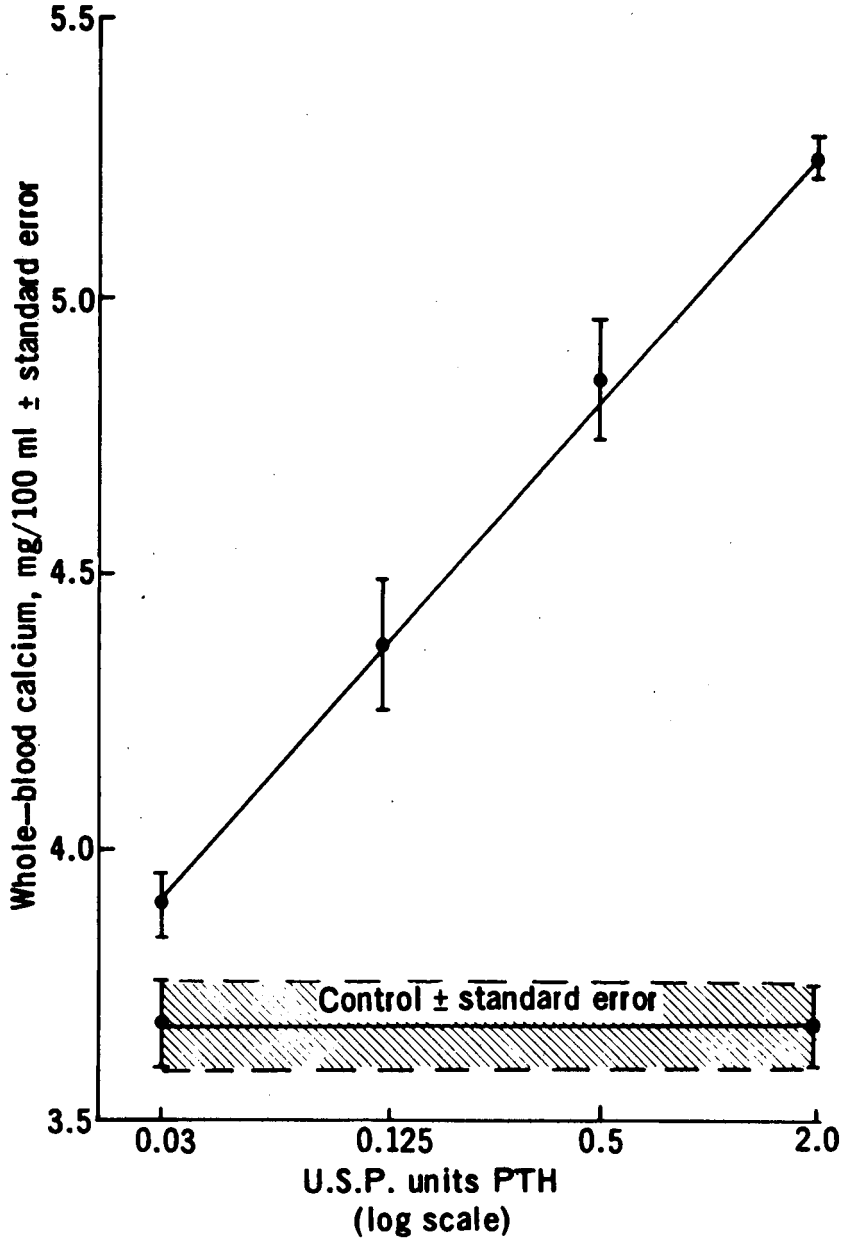

Figure 9-1.- Typical assay results.

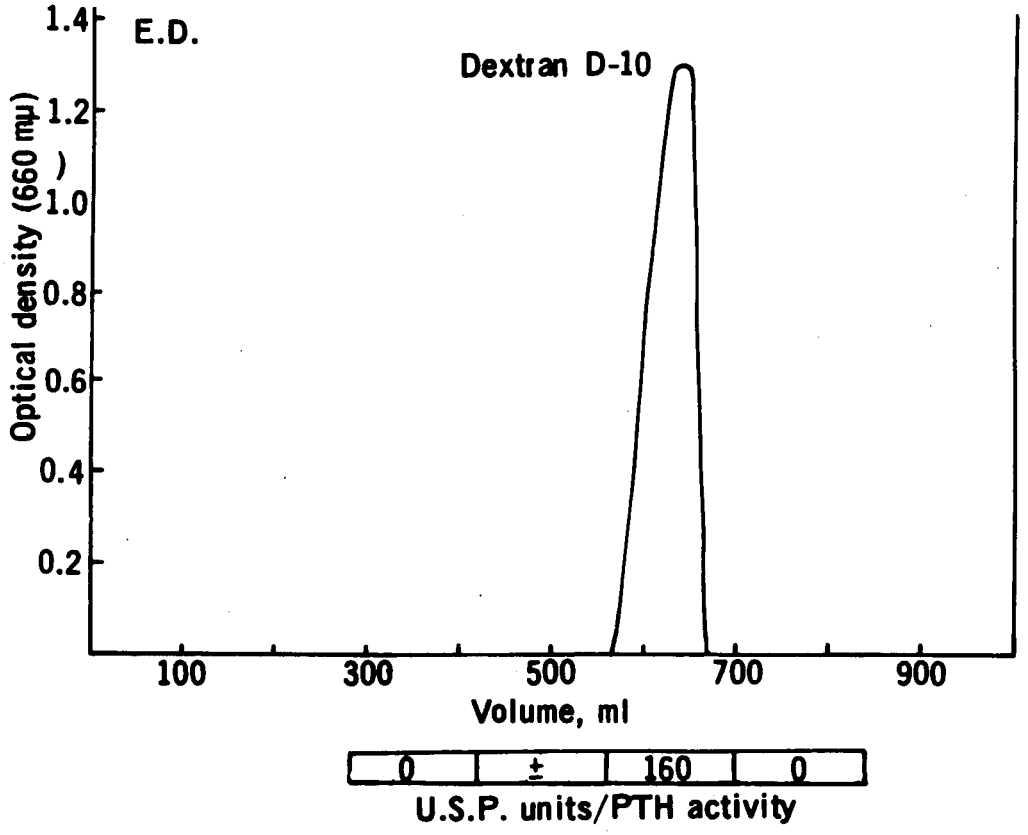

Figure 9-2.- Uremic urine PTH activity and Dextran of average molecular weight of 9400 on Sephadex G-50.

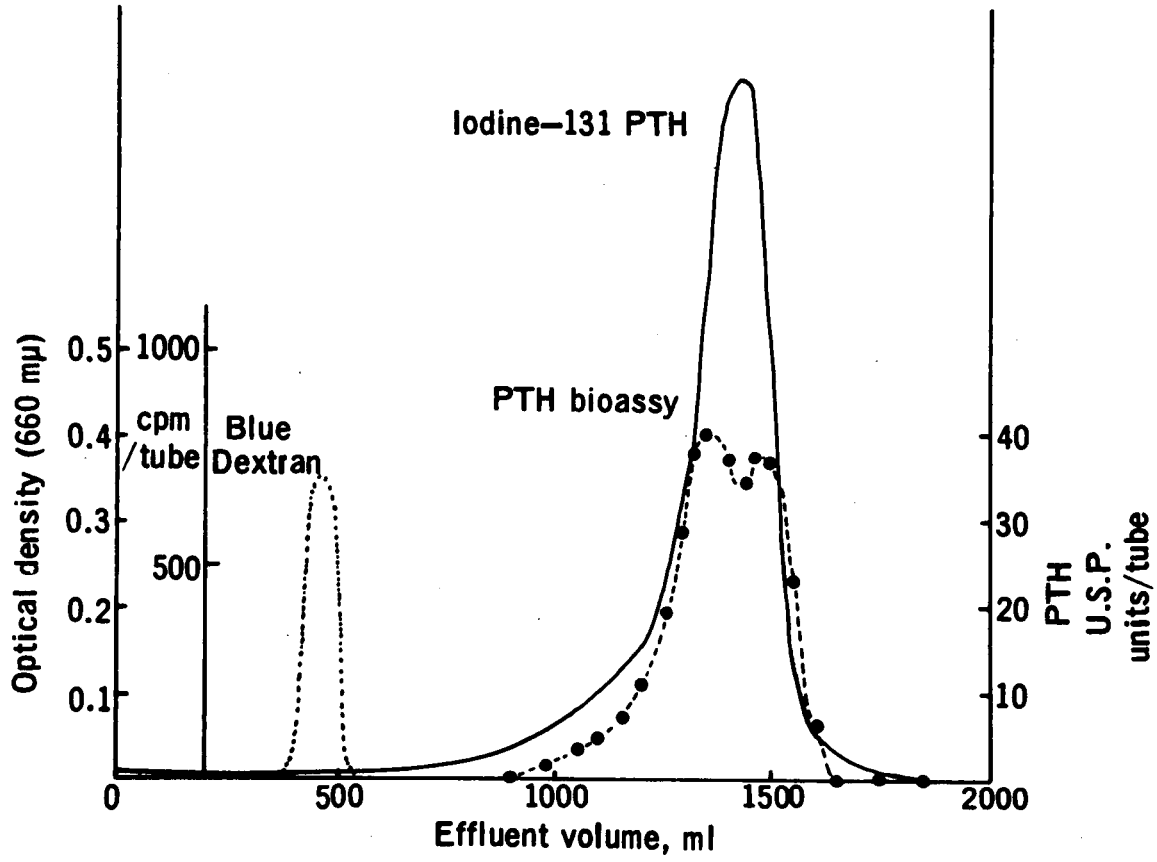

Figure 9-3.- Results of column chromatography of added beef iodine-131 PTH and urine from a patient with uremic osteodystrophy.

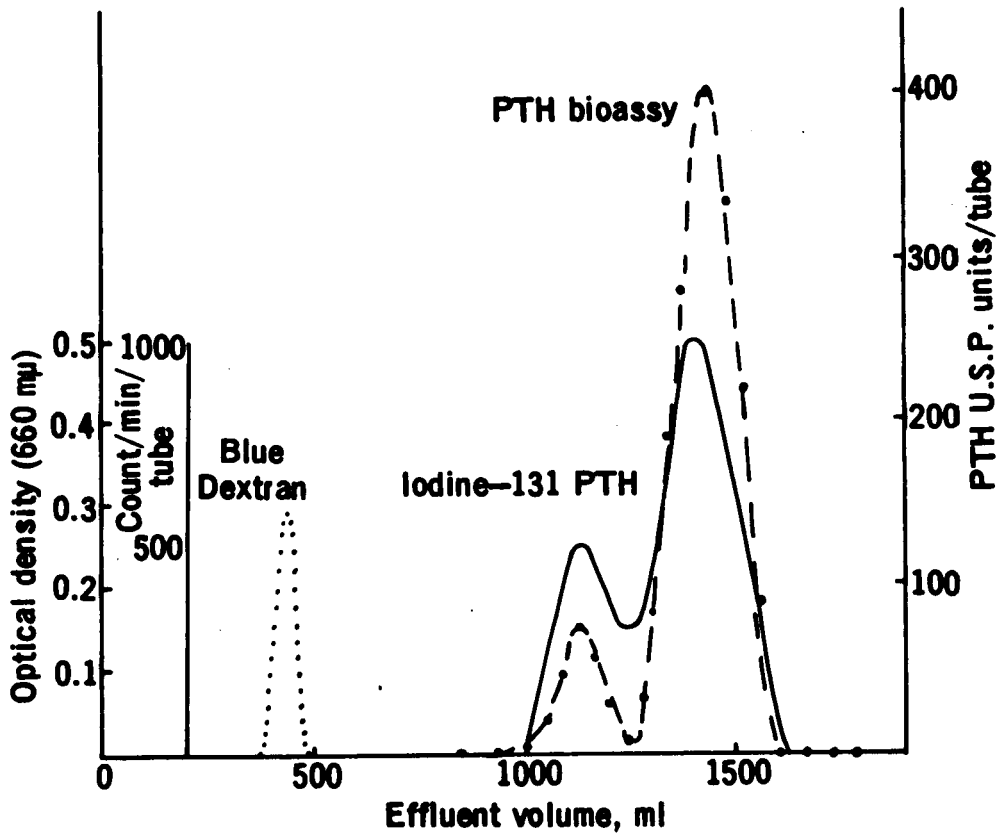

Figure 9-4.- Results of column chromatography of added beef iodine-131 PTH and urine from a patient with parathyroid adenoma.

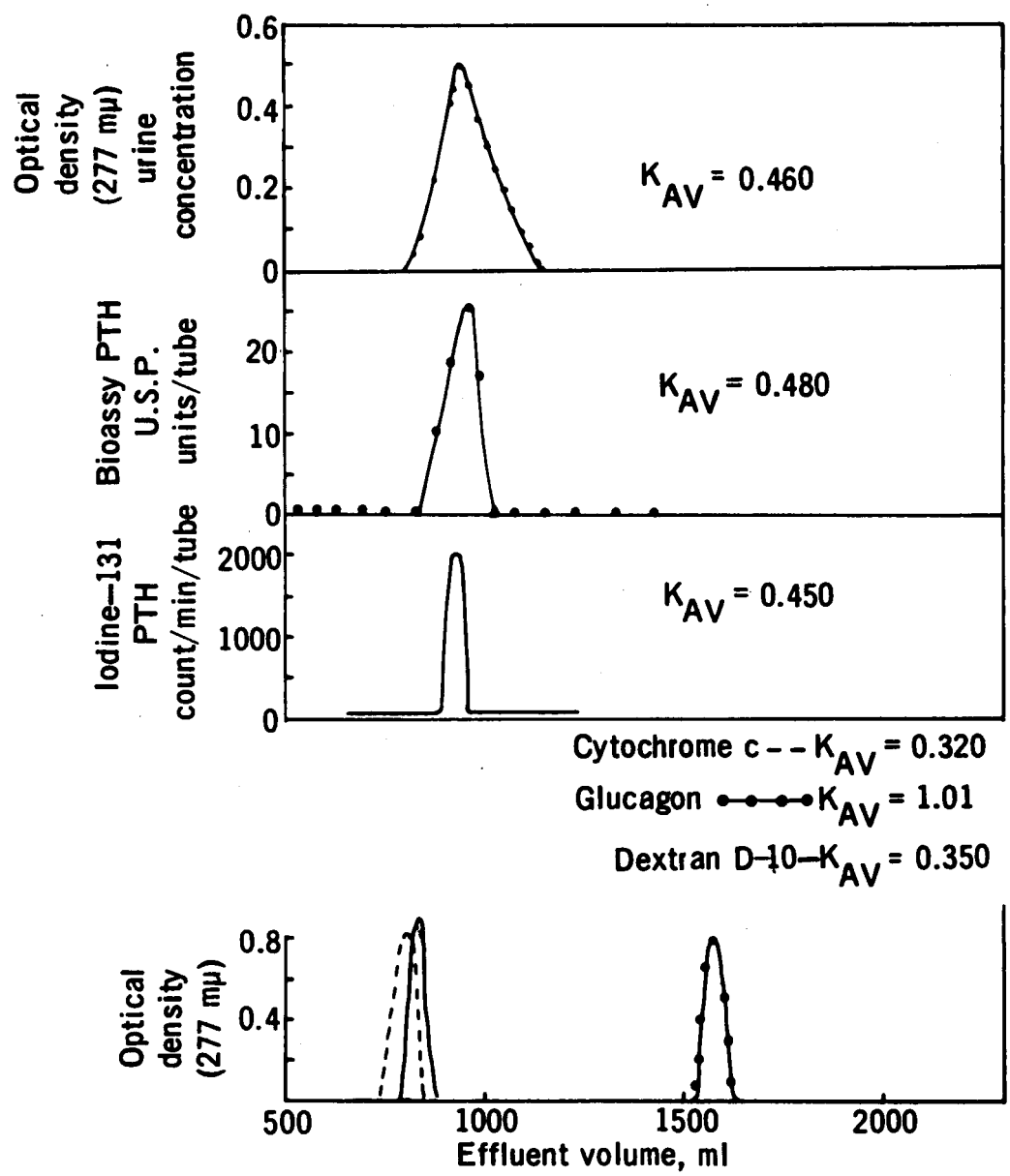

Figure 9-5.- Characteristics of urine PTH from a patient with uremic osteodystrophy on Bio-Gel P-10.

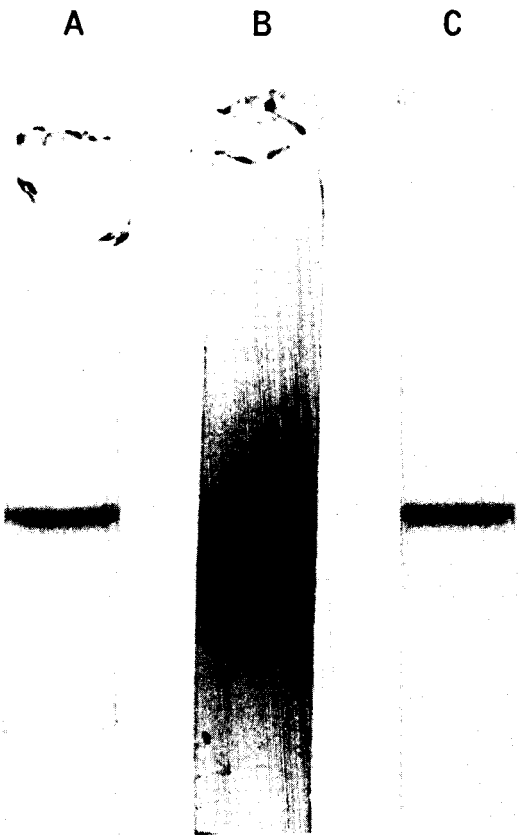

Figure 9-6.- Results of disc-gel electrophoresis.

10. PRELIMINARY RESULTS OF ACTH RADIOIMMUNOASSAY

By Bonnalie O. Campbell, Ph. D.*

INTRODUCTION

It is most significant that an endocrine meeting has been held within the Lunar Receiving Laboratory at the Manned Spacecraft Center to review the NASA-sponsored biomedical work relating to man in space and to discuss the current programs that will extend the knowledge obtained from the Gemini and Apollo flights to later Apollo and Skylab missions. It is encouraging to note the formation of a devoted group of scientists who have, within only a few years, developed research programs of a highly applied nature that are directed to the specific problems encountered in normal space flight and who continue to expand this scientific base during a period of general public apathy.

Several years ago, Dr. W. W. Kemmerer, Jr., and others at the Manned Spacecraft Center convened a meeting to discuss a proposed protocol for the biological testing of the lunar samples and possible facilities that might be needed for such a program. From the initial meeting, there evolved a year of intensive work that culminated in a biological protocol for what was then called the Lunar Sample Receiving Laboratory. The comprehensive protocol (ref. 10-1) was submitted to NASA by the staff of the Baylor College of Medicine; more than 100 consultants from the biological sciences assisted in this preliminary work.

DISCUSSION

The quarantine biological testing was to be a three-point program. The first point involved the establishment of preflight and postflight microbial profiles of the crew and spacecraft. The other two points were directly concerned with the lunar samples; to wit, methods of culturing the samples using conventional microbiological techniques, and of exposing selected plants and animals to the lunar materials for varying periods of time.

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These tests were designed with the objective of detecting any replicable extraterrestrial agent that might constitute an immediate hazard to the earthly biosphere or, in the case of the crew, of detecting any indigenous earth form that might have undergone alterations and become pathological during the course of extended space flight. This proposed protocol was one of the first serious attempts made by an outside group of scientists to draw attention to the fact that man as a biological entity was an integral component of a specific mission and should be dealt with as such and that, consequently, man was not uniquely exempt from the close scientific scrutiny to which the lunar samples and spacecraft are subjected after return to earth.

During this period of study, which was devoted to formulating concepts of testing and assembling laboratory procedures to maintain the biosphere free of potentially harmful pathogens, the recurring question arose — what is being done to prepare man in his immediate environment for prolonged existence outside this protective terrestrial capsule? Admittedly, carefully executed programs and chamber studies of controlled lighting, diet, and work and rest schedules had been conducted as well as microbial studies performed under conditions of high oxygen pressure.

But the question remained as to whether these studies provided sufficient data on which specific recommendations could be made concerning man's internal and immediate external environment so that such diverse parameters as man's problem-solving ability, depth-perception, sleep-wake cycles, and so forth could be protected and maintained functional. Another question was what valid recommendations could be made to afford protection against stress and to establish optimal light and sound regimens of specific intensity, duration, and frequency. These and other like questions can be answered thoroughly and recommendations can be made only when research programs of a pragmatic nature (such as the subject of this symposium) are given support.

It is likely that a study of human adrenal functions may provide some answers to the questions of the effects of space flight on human physiology. Obviously, the problems encountered in manned space flight thus far — erythrocyte-mass changes, bone demineralization, cardiovascular deconditioning — may share some common denominator in which pituitary-adrenal function is of direct or indirect significance. No doubt exists that the hypothalamic-pituitary-adrenal axis has a central integrative function in homeostasis and in conditioning the human body to various environmental stimuli that are stressful.

Recently, it has been suggested that, for the early detection of disease, an examination of the adrenal-pituitary axis might indicate the initiation of a possible diseased state before the onset of clinical symptoms. An assay for the adrenocorticotrophic hormone (ACTH) would

thus be of value not only in serving as an index for the early detection of disease, but also in defining the stressed state in mission-oriented studies. This assay would be of considerable importance in relation to final crew selection for long-duration missions, because ascertaining the stress level would prevent the commitment of stressed crewmen who had not exhibited any signs of preflight illness.

Because of the central role of the hypothalamic-pituitary-adrenal system in the organism's response to stress, a developmental program has been initiated to assess adrenal control mechanisms in human and nonhuman primates to achieve a better understanding of how levels of pituitary hormones affect human behavior under various conditions.

To accomplish these objectives, the clinical analytic technique chosen was the radioimmunoassay for ACTH in peripheral plasma, using the fundamental assay of Yalow and Berson (ref. 10-2) as modified by R. A. Donald (refs. 10-3 and 10-4). This assay was originally developed in a metabolic study for ^{131}I-insulin injected intravenously in man. By the use of this basic technique, plasma concentrations of numerous peptide hormones in the picogram (10^{-12}) or nanogram (10^{-9}) range can now be determined immunochemically. Although bioassays exist for a number of these hormones, some are not suitable for routine clinical analysis. In addition to being inherently complex and variable, these standard assays require large quantities of blood. The radioimmunoassay obviates these problems.

The radioimmunoassay for ACTH, although offering the potential of great sensitivity and specificity, has not been perfected as a routine clinical-laboratory technique, primarily because all three key components of the assay are not as yet commercially available. These components are (1) the highly purified ACTH essential in the preparation of reference standards, (2) antisera with high titre and specificity, and (3) the iodine-labeled hormone acting as a tracer.

Highly purified ACTH suitable for standardization or routine radioiodination is very difficult to obtain. Although active ACTH preparations were first isolated from the sheep and pig in the early 1940's (refs. 10-5 and 10-6), few sources of purified hormone are readily available to investigators at present. Much of the highly purified material is circulated as a gift from one laboratory to another. The ACTH required for general immunization purposes, however, does not need to be highly purified and can be obtained from a commercial supplier. Antibodies formed in response to contaminating peptides do not interfere in the assay.

Theoretically, in any assay only one species of corticotrophin should be used; that is, in the radioimmunoassay for human ACTH, the standards,

labeled hormone, and the ACTH used for immunization should all be human ACTH, either natural or synthetic (ref. 10-7). This requirement is based on the consideration of specie structural differences in the amino-acid sequence from positions 25 to 33 as numbered from the N-terminal end of the polypeptide chain. In practice, observing these conditions is very difficult, because human ACTH is not readily available.

Both ^{125}I and ^{131}I are commercially available for use in the preparation of the labeled hormone. The ^{131}I-labeled hormone can be prepared with a higher specific activity than ^{125}I, but it has a half-life of only approximately 8 days. In contrast, ^{125}I has a considerably longer half-life of 56 days, which eliminates the need for more frequent iodinations.

The iodination procedure that has been used in the Baylor College of Medicine laboratories is Landon's modification of Hunter's and Greenwood's methods (refs. 10-8 to 10-10). Chloramine-T is used as the oxidizing agent to facilitate iodination, and the reaction is terminated by the addition of sodium metabisulfite. Despite the ease and rapidity of the iodination, the procedure sometimes yields unpredictable results and an occasional inexplicable failure is to be anticipated. Experience has shown differences in the responses of batches of chloramine-T and isotopes to the reaction.

Production of highly specific antibodies to ACTH is perhaps the most critical aspect of the radioimmunoassay for the hormone, because several animals must be immunized before an antiserum of suitable titre is developed. The reason several animals must be immunized is the tremendous biological variation in the antigen-antibody response. Recent private communications suggest that an independent research laboratory in Kent, England, may be marketing a suitable antiserum in the near future.

As a part of the current study, a program of antibody production has been undertaken, involving the immunization of rabbits, guinea pigs, and chickens. In the first series of immunizations, Duracton^{R} was administered subcutaneously at a 1:1 ratio with Freund's complete adjuvant. Duracton^{R} contains a porcine ACTH combined with carboxymethyl cellulose, which serves to slow the release of the hormone and also protects the hormone against immediate inactivation. Subsequent booster injections have been coupled with Freund's incomplete adjuvant. Metyrapone, an 11 β-hydroxylase inhibitor, was given intramuscularly, although this precaution to inhibit the final step in corticosteroid synthesis is not always considered essential to obtaining satisfactory antibody titres.

A typical titration curve for preliminary antisera screening is shown in figure 10-1. The assay tubes contained serial dilutions of rabbit plasma, 1 percent (weight to volume) citrated lyophilized horse plasma (Pentex), and repurified ^{125}I-labeled p-ACTH; the dilutions, of from 1:50 to 1:5000, were allowed to incubate for 3 days at 4° C. The controls were rabbit 6, horse plasma, horse plasma with Middlesex 1/500, and all rabbit dilutions of 1:5000 with Middlesex 1/500 added. The Middlesex antiserum, one of known sensitivity and specificity, was obtained from R. A. Donald, presently at Princess Margaret Hospital, Christchurch, New Zealand. Fair percent binding was found at final antibody dilution of 1:50 for rabbit 1, which suggests continuation of the immunization regimen.

A typical variable response that can be expected with an antibody-titration curve is shown in figure 10-2. The plasma samples were collected approximately 4 weeks after the data shown in figure 10-1 were derived. The percent of bound labeled hormone had increased for all dilutions of rabbit 1 antiserum. However, a uniform decrease in binding of all dilutions of rabbit 2 antiserum was noted. Interestingly, rabbit 2 later died after receiving a booster injection.

The increasing antibody production shown by plasma taken from rabbit 1 is illustrated in figure 10-3. After a satisfactory titre has been obtained, the next step in antisera screening is establishing standard curves to determine the sensitivity and specificity of the antiserum in question.

The antibody-titration curves of the first bleeding of six chickens are shown in figure 10-4. The protocol for injection was essentially the same as for the rabbits, except that the chickens were injected in the comb, rather than subcutaneously. Only three of the four chickens injected show an antibody response. None of the ^{125}I-αp-ACTH was bound by plasma obtained from chicken 5 and from the controls (0 percent binding). A program of immunization of guinea pigs has been initiated, but screening for antibody production at this time would be premature.

The method of radioimmunoassay used in this program is the one described by R. A. Donald (refs. 10-3 and 10-4), which he used while a fellow at the Baylor College of Medicine. Aliquots of the ^{125}I-labeled hormone are purified before each use. The hormone is adsorbed on silicic acid and the undamaged hormone is then eluted with a solution of acetone and acetic acid. The ACTH is adsorbed from plasma by silicic acid using a similar method. This technique is necessary because unextracted plasma levels are below the levels of sensitivity of the method currently used.

Several techniques have been devised to separate the bound antibody from the unbound fraction, such as the use of talcum powder (hydrated

magnesium silicate), ion-exchange resins, a second antibody, and other methods. The preferred technique, also used by Donald, involves dextran-coated charcoal to which the unbound ACTH is adsorbed. The total incubation period is for 5 days at 4° C.

A typical standard curve of an extracted assay for human plasma using the assay method described is shown in figure 10-5. The percent of labeled hormone bound to antibody appears to be slightly lower than the values normally obtained with an extracted assay, possibly because of the age of the αh-ACTH used as the reference standard. By use of the extraction method, values have been obtained for circulating plasma levels that are comparable to those reported for normal subjects exhibiting a diurnal variation ranging from 0 to 80 pg/ml (ref. 10-11); higher levels for plasma taken during periods of stress have been observed.

CONCLUDING REMARKS

The purpose of using the radioimmunochemical assay for ACTH in this research program is to establish the capability for routine analysis of human plasma levels of corticotrophin while performing three ground-based studies.

1. Circulating plasma levels of ACTH will be measured directly to establish base-line values of normal ACTH secretion obtained under various nonstress situations. In establishing these base-line values, parameters such as sex, time of day, work-rest cycles, light-dark cycles, and so forth will be considered. These parameters may be of considerable significance, because sex, light-dark cycles, and time of day have been reported to affect ACTH levels in the rat (ref. 10-12).

2. Normal ACTH values obtained under restful conditions will be analyzed in real time for circadian variation and correlated with available cortisol values that would indicate an underlying circadian rhythm in the total pituitary-adrenal axis. Retiene and others (ref. 10-13) have provided evidence for the long-suspected diurnal variation of ACTH secretion in the nonstressed human. Adrenal corticosteroids are known to exhibit rhythms of a diurnal or circadian nature. Release of these steroids from adrenal corticol tissues is affected by the circulating plasma levels of ACTH.

3. The limits of the radioimmunochemical assay in determining the stressed state as opposed to base-line or nonstress values will be considered, as well as the effect of stress situations on circulating plasma levels. Any possible disruption or suppression of the circadian rhythm induced by stress conditions will be evaluated, as well as the length of time necessary to return to the normal resting value and periodicity.

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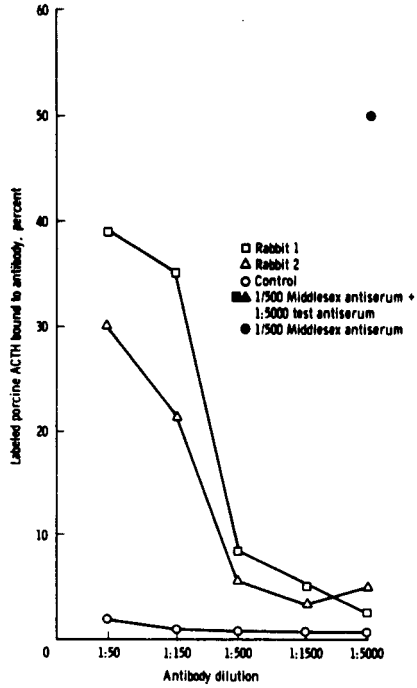

Figure 10-1.- Typical titration curve for preliminary antisera screening.

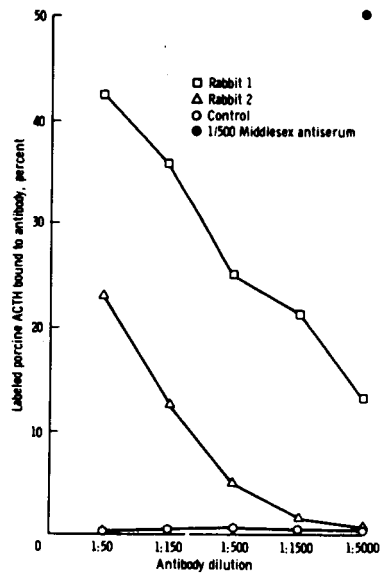

Figure 10-2.- Typical variable response expected in antibody-titration curve.

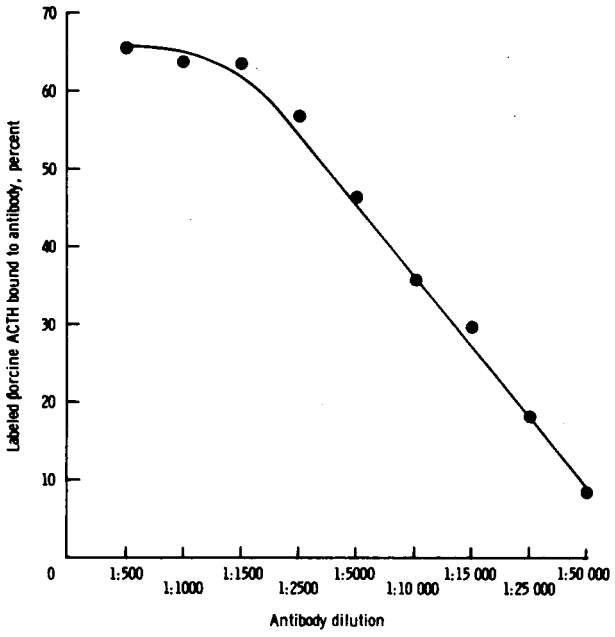

Figure 10-3.- Increasing antibody production shown by plasma from rabbit 1.

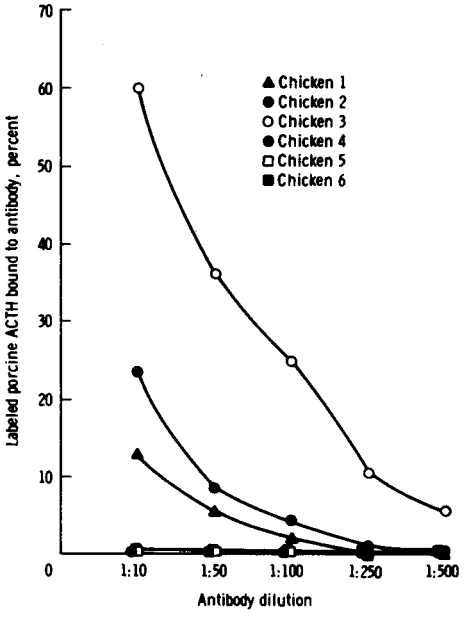

Figure 10-4.- Antibody-titration curves of initial bleeding of six chickens.

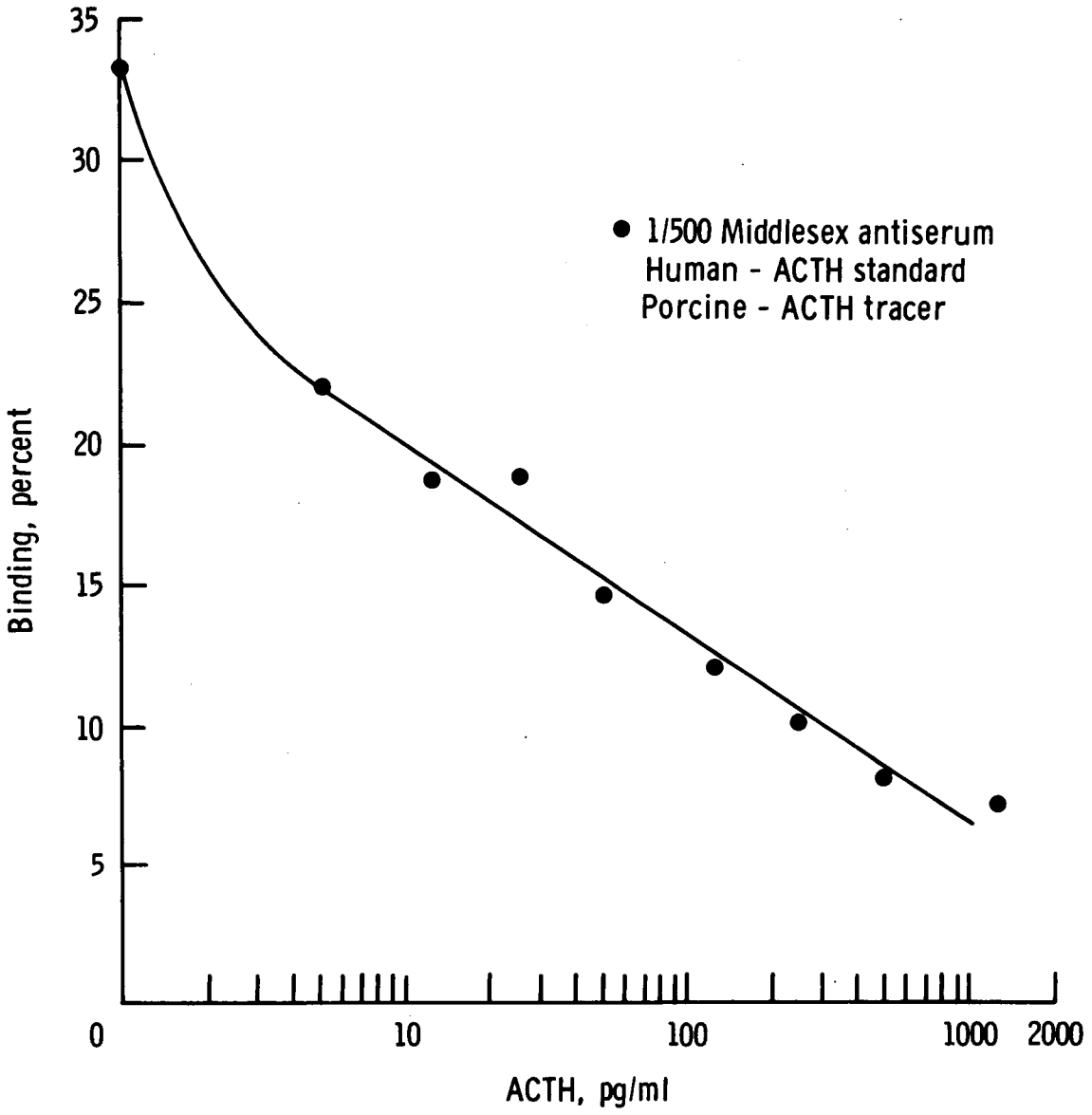

Figure 10-5.- Typical standard curve of extracted assay for human plasma.